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Development and Evaluation of Novel Baricitinib Formulation for Psoriasis Treatment

Roya Mohammadi Mey Abadi

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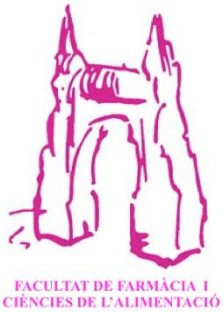

Development and Evaluation of Novel Baricitinib Formulations for Psoriasis Treatment

TESIS DOCTORAL

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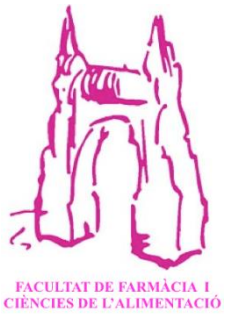

BIOTECHNOLOGY DOCTORAL PROGRAM

Development and Evaluation of Novel Baricitinib Formulations for Psoriasis Treatment

Report Presented by

Roya Mohammadi Mey Abadi

To Qualify for the Title of

PhD in Biotechnology

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CONTENTS

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After a great and wonderful journey, when I look back, I see what a long path full of hardships and ups and downs I have traveled. After twenty-four years of academic pursuit across various schools and faculties, I am both delighted and excited. I have reached a new scientific level in my scientific life.

This will be the beginning of my new professional life. Doctoral study opened a new mental window for me. I would like to convey my sincere gratitude and appreciation to everyone who has supported and accompanied me throughout this journey.

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I want to extend my deepest gratitude to ***Professor. Ana Cristina Calpena Campmany*** *my tutor and the main director of my doctoral project, for her invaluable guidance, the many*

opportunities she provided for my growth and learning, and her patience and motherly care, nurturing support over the years. I am truly appreciative of all she has done.

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At the end of this thesis is dedicated to:

My father

My mother

My brothers

My grandfather, my mother's father, passed away a few years ago, but while he was alive, he was one of my main encouragers in education and he wished that one day I would get a doctorate in a scientific field. He is happy because of me in the other world.

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And maybe it is arrogant to present this thesis to myself, because of all the sufferings, hardships, bitterness and sweetness, disappointments and hopes, failures and victories, lack of sleep, staying up all night, being far away, grudges and cries, sadness, laughter, and happiness that I had.

I am ending this chapter of my life. I am and will be forever indebted to all of you who played a huge role in my progress in achieving this success.

Roya Mohammadi Mey Abadi

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ABBREVIATIONS

AA: Alopecia Areata

AD: Atopic Dermatitis

AICARTase: Aminoimidazole Carboxamide Ribotide Transformylase

APC: Antigen-Presenting Cell

API: Active Pharmaceutical Ingredient

BCT: Baricitinib

BSA: Body Surface Area

CD2: Cluster of Differentiation 2

CPK: Creatine Phosphokinase

CYP3A4: Gene is a cytochrome P450 family 3 subfamily A member 4 enzyme

DALY: Disability-Adjusted Life Year

DEGEE: Diethylene Glycol Monoethyl Ether

DLQI: Dermatology Life Quality Index

ECETOC: European Center for Toxicology and Toxicology of Chemicals

EFSA: European Food Safety Agency

EMA: European Medicines Agency

FDA: Food and Drug Administration

GBDS: Global Burden of Disease Study

GPA: Global Psoriasis Atlas

HLA: Human Leukocyte Antigen

HPLC: High-Performance Liquid Chromatography

IFNs: Interferons

IL: Interleukins

IMQ: Imiquimod

INF-γ: Interferon gamma

IVRT: In Vitro Release Testing

JAK: Janus kinase

LFA: Lymphocyte Function-associated Antigen

LOD: Limit of Detection

LOQ: Limit of Quantitation

MHC: Major Histocompatibility Complex

NB-UVB: Narrow-Band Ultraviolet B

OAT3: Organic Anion Transporter 3

OECD: Organization for Economic Co-operation and Development

PASI: Psoriasis Area and Severity Index

PK: PharmacoKinetics

PSA: PSoriatic Arthritis

PSORS1: Psoriasis Susceptibility 1 locus

PUVA: Psoralen and UltraViolet A

RA: Rheumatoid Arthritis

RE%: Relative Error or Percent deviation

SC: Stratum Corneum

SCH: Stratum Corneum Hydration

STAT: Signal Transducer and Activator of Transcription proteins

TEWL: Trans Epidermal Water Loss

TNF: Tumor Necrosis Factor

TSLP: Thymic Stromal Lymphopoietin

TYK2: Tyrosine Kinase 2

USEPA: US Environmental Protection Agency

UV: Ultraviolet-Visible

WHO/IPCS: World Health Organization International Program on Chemical Safety

WHO: World Health Organization

ABSTRACT

This thesis focuses on developing a baricitinib-based formulation to manage psoriasis. The research was structured around two primary objectives. The first objective explored the solubility of baricitinib in various excipients and media as a part of the pre-formulation phase. It aimed to select suitable excipients for a lipid-based formulation, study drug uptake through different tissues, and evaluate the efficiency of drug extraction from those tissues. Additionally, an analytical method to quantify baricitinib in different samples is developed and validated within this framework.

Baricitinib, a poorly soluble drug, presents challenges in formulation, making solubility an important factor to address during the preformulation stage. Solubility significantly impacts bioavailability, especially in oral and topical applications. By understanding its solubility, we could select the most appropriate excipients and solvents to enhance drug absorption and optimize formulation stability. The solubility studies in this thesis aim to ensure that the drug remains stable over time in its solvent to prevent precipitation or degradation, both of which are common formulation challenges.

The first objective was successfully addressed in a published article, "Assessing the Solubility of Baricitinib and Drug Uptake in Different Tissues Using Absorption and Fluorescence Spectroscopies." This article tackled baricitinib's low water solubility, testing its solubility in various solvents, including oils and permeation enhancers like Transcutol®, N-ethyl pyrrolidone, and others. Among these, Transcutol® and N-ethyl pyrrolidone emerged as the most effective solvents for dissolving baricitinib. Transcutol,

a widely used biocompatible solvent and permeation enhancer in pharmaceutical and cosmetic products, significantly improved baricitinib's solubility, facilitating higher drug concentrations.

Following these solubility studies, drug uptake and recovery tests were conducted to assess how much baricitinib penetrated various tissues and evaluate drug extraction methods' effectiveness. Human and porcine tissues, such as skin, buccal, sclera, cornea, sublingual, and vaginal mucosa, were exposed to baricitinib in Transcutol. The concentration of baricitinib absorbed by these tissues was measured using absorption spectroscopy. The drug was then extracted from these tissues via sonication, and the recovery rates varied depending on the tissue type. For example, the buccal mucosa showed the highest uptake of baricitinib, while the cornea and sublingual mucosa had the best recovery rates.

In addition to the solubility and uptake studies, analytical methods for determining baricitinib concentration were developed, employing absorption and fluorescence spectroscopy. Absorption spectroscopy was effective for measuring higher concentrations of the drug, while fluorescence spectroscopy was suited for detecting lower concentrations. These complementary methods were validated and found to be simple, cost-effective, and suitable for routine analysis in pre-formulation studies.

The second objective of the thesis was to develop and evaluate a lipid-based formulation of baricitinib for topical use, particularly for psoriasis treatment. The selected formulation, BCT-OS, included Transcutol P® as a primary solvent for its strong solubilizing capacity and skin compatibility, along with secondary oily vehicles like Labrafac Lipophile, and Lauroglycol 90, Surfadone

LP 100 was used as a permeation enhancer to aid drug absorption through the skin. The final formulation was optimized for stability, drug content, and pH, with a pH of 5.60, making it suitable for topical application.

The in vitro and ex vivo studies showed that around 80% of the drug was released from the formulation, with most of the drug retained in the skin layers rather than passing through them. This suggests that the formulation is effective for localized treatment, limiting systemic absorption and potential side effects. The high retention of baricitinib in the skin is essential for treating psoriasis, where prolonged contact with the affected area is necessary.

BCT-OS was tested in a psoriasis-induced model in mice, demonstrating a reduction in key symptoms such as skin thickness, erythema, and edema. The formulation also improved skin hydration and barrier function, addressing psoriasis-induced dehydration and dryness. Histological analysis revealed a decrease in inflammation, indicating the formulation's effectiveness as an anti-inflammatory treatment.

Tolerance studies confirmed that BCT-OS improved skin barrier function without causing irritation or inflammation, supporting its safety for long-term use. Overall, the baricitinib-based lipid formulation developed in this thesis shows strong potential for managing psoriasis, providing localized treatment with minimal systemic effects, and promoting skin hydration, and barrier function.

RESUMEN

Esta tesis se centra en desarrollar una formulación de baricitinib para tratar la psoriasis. El trabajo se estructuró en dos objetivos principales. El primero se enfocó en estudiar la solubilidad de baricitinib en diferentes excipientes y medios, como parte de la fase de preformulación. El objetivo era seleccionar los excipientes adecuados para una formulación lipídica, estudiar la absorción del fármaco en diferentes tejidos y evaluar la eficacia del método de extracción del fármaco de dichos tejidos. Además, se desarrolló y validó un método analítico para cuantificar baricitinib en diversas muestras.

Dado que baricitinib es un fármaco de baja solubilidad, mejorar su solubilidad es clave para aumentar su biodisponibilidad, especialmente en aplicaciones orales y tópicas. El estudio de la solubilidad permitió seleccionar los mejores excipientes y solventes, mejorando la absorción y estabilidad de la formulación.

El primer objetivo se logró con la publicación de un artículo titulado "Evaluación de la Solubilidad de Baricitinib y la Absorción en Diferentes Tejidos Usando Espectroscopías de Absorción y Fluorescencia". Este trabajo probó la solubilidad de baricitinib en varios solventes, destacando Transcutol y N-etil pirrolidona como los más efectivos. Transcutol, un solvente biocompatible y mejorador de la permeabilidad, aumentó significativamente la solubilidad de baricitinib, permitiendo mayores concentraciones de fármaco.

Posteriormente, se realizaron estudios de absorción y recuperación del fármaco en tejidos humanos y porcinos, como la piel, mucosa bucal, sublingual, córnea y vaginal. La mucosa bucal mostró la mayor absorción de baricitinib, mientras que la córnea y la mucosa sublingual tuvieron las mejores tasas de recuperación. Los métodos analíticos validados, basados en espectroscopías de absorción y fluorescencia, resultaron ser simples, económicos y adecuados para estudios rutinarios de preformulación.

El segundo objetivo fue desarrollar y evaluar una formulación lipídica de baricitinib para uso tópico en psoriasis. La formulación, llamada BCT-OS, utilizó Transcutol P® como solvente principal por su capacidad de solubilización y compatibilidad con la piel. Los estudios in vitro y ex vivo mostraron que la mayor parte del fármaco se retiene en las capas de la piel, lo que indica su efectividad para el tratamiento localizado.

Finalmente, en un modelo de psoriasis en ratones, BCT-OS redujo síntomas clave como el grosor de la piel, el eritema y el edema. También mejoró la hidratación y la función de la barrera cutánea, demostrando su potencial para el tratamiento de la psoriasis con efectos sistémicos mínimos y buena tolerancia.

INTRODUCTION

Introduction

1. Psoriasis

Psoriasis is a chronic, multifactorial inflammatory disease that significantly impacts the quality of life of those affected. It is characterized by erythematous-desquamative plaques, though it may present in a variety of clinical manifestations. Despite its prevalence, the exact etiology of psoriasis remains elusive., involving a complex interplay of genetic., immunological., and environmental factors. Despite the availability of various treatments, many patients continue to experience inadequate relief, adverse side effects, or a decline in efficacy over time. This underscores the urgent need for innovative therapeutic approaches.

1.1. Prevalence and Epidemiology

In 2014, the World Health Organization (WHO) recognized psoriasis as a non-communicable disease and highlighted the distress related to misdiagnosis., inadequate treatment., and stigmatization of this disease and emphasized the need to better understand its global burden. To address this need, the Global Psoriasis Atlas (GPA) was created to conduct more research on the prevalence and incidence of psoriasis worldwide, aiming to improve access to care (1-2).

Accurate determination of the age of onset of psoriasis is problematic, as studies that do so typically rely on a patient's recall of the onset of lesions or determine the onset of psoriasis can often be subtle, with minimal symptoms that may go unnoticed for years before a patient seeks consultation. Data based on patient recall can be inaccurate., determining onset based on the first visit to a physician could underestimate the time of disease occurrence., as minimal disease with a bimodal age of onset has been identified in several large studies. The age of onset of the first manifestation of psoriasis is on average between 15 and 20 years., and the second peak occurs at the age of 55-60 *(3-6).*

Psoriasis is common globally, affecting an estimated 60 million people. It affects men and women of all ages, ethnicities, and backgrounds or places of residence. and it starts earlier in women and those with a family history.

Published data on the prevalence of psoriasis vary from 0.09% to 11.4%, influenced by factors such as age, gender, geography, ethnicity, and genetic and environmental factors (1). In most developed countries., the prevalence is between 1.5 and 5%. There is also evidence to suggest that the prevalence of psoriasis may be increasing (2).

Its age of onset shows a bimodal distribution with peaks at 30-39 years and 60-69 years in men, and 10 years earlier in women *(1).*

Over the past decade, research into the epidemiology of psoriasis has increased., particularly in countries where estimates were previously unavailable. Recent studies have often been derived from resources with

better quality data, resources, such as large databases of electronic health data that are more nationally representative than previous studies (1).

A systematic review has shown that epidemiological data on the incidence of psoriasis are limited., and studies have been conducted mainly in Europe and North America. The findings showed consistency between studies in the bimodal pattern of the age of onset (1). No agreement was found regarding sex-specific differences or trends over time. Disease prevalence was highest in high-income regions such as Australasia, Western Europe, Central Europe, and North America (Figure 1). However, the largest adult patient populations affected by psoriasis live in the United States of America (US), India, and China, followed by Germany, Brazil, France, and the United Kingdom. Both the incidence and prevalence of psoriasis have a strong association with age, it was seen that the disease was less frequent in children and more frequent in adults(1) (Table 1).

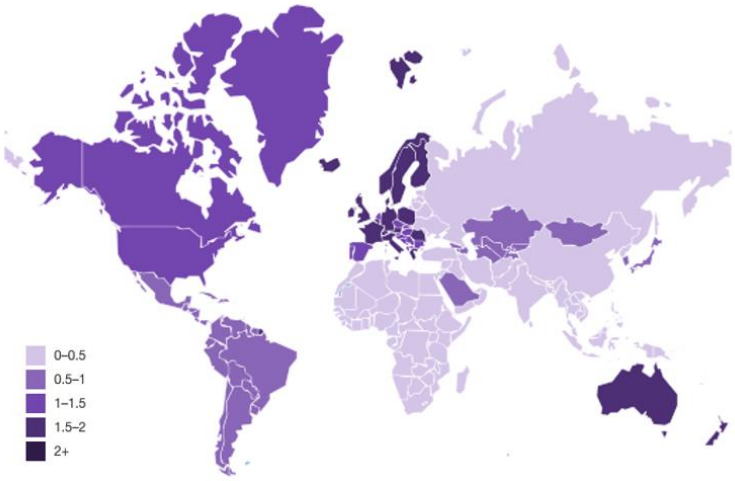

Figure 1. The world map of the prevalence of psoriasis shows that Australasia., Western Europe., Central Europe., and North America are the populations with the highest prevalence of the disease (1-2.,7).

The Global Burden of Disease Study (GBDS), 2010 (8) aimed to measure disability or health care needs due to various conditions. One of the metrics commonly used for this purpose is the disability-adjusted life-year (DALY), which represents the loss of one year of healthy life (2).

The GBDS estimated that psoriasis accounted for 5.6 million all-age (DALYs) in 2016, at least three-fold that of inflammatory bowel disease *(9).*

Hensler and Christophers studied 2147 patients and reported two clinical manifestations, type I and type II, defined by dichotomous age at onset. Type I psoriasis begins at or before the age of 40, while Type II psoriasis begins after the age of 40. Type I disease accounts for more than 75% of cases *(6).* Patients with early onset, or type I psoriasis, tended to have more relatives affected and more severe disease than this classification highlights the distinction in the age of onset among patients., particularly those with later onset or Type II psoriasis.

An estimated 60 million people have psoriasis worldwide., with country-specific prevalence varying between 0.05% of the general population in Taiwan and 1.88% in Australia *(1,7).* It is more common in high-income areas and those with older populations *(1).* The prevalence of psoriasis is low in certain ethnic groups such as the Japanese and may be absent in Aboriginal Australians *(10),* and Indians from South America *(11).*

81% of countries worldwide have no information on the epidemiology of psoriasis. The distribution of psoriasis is uneven across geographic regions, and it is more prevalent in high-income countries and areas with larger populations. The estimates provided can help guide countries and the international community in making public health decisions about the

appropriate treatment of psoriasis and assessing its natural history over time (1).

Table 1. List of the 10 countries with the highest prevalence of psoriasis in the world (7).

Country	Region	Overall	Adults	Children
Australia	Australasia	1.88	2.38	0.27
Norway	Western Europe	1.86	2.36	0.26
Israel	Western Europe	1.81	2.28	0.26
Denmark	Western Europe	1.79	2.26	0.25
Romania	Central Europe	1.77	2.24	0.25
Germany	Western Europe	1.74	2.2	0.25
Sweden	Western Europe	1.66	2.1	0.23
Poland	Central Europe	1.63	2.06	0.23
Italy	Western Europe	1.58	2	0.22
New Zealand	Australasia	1.58	1.99	0.22

According to the (GPA), Spain ranks 46th in psoriasis prevalence. In a population of 46.35 million inhabitants, there are 514,480 people affected by psoriasis, with a prevalence of 1.11% overall, 1.4% in adults, and 0.16% in infants (7).

Psoriasis is generally considered equally frequent in both sexes., though, some studies report a higher prevalence in men(12) (Figure 2). However, these findings are not statistically significant and require further research., particularly regarding genetic and environmental factors (1).

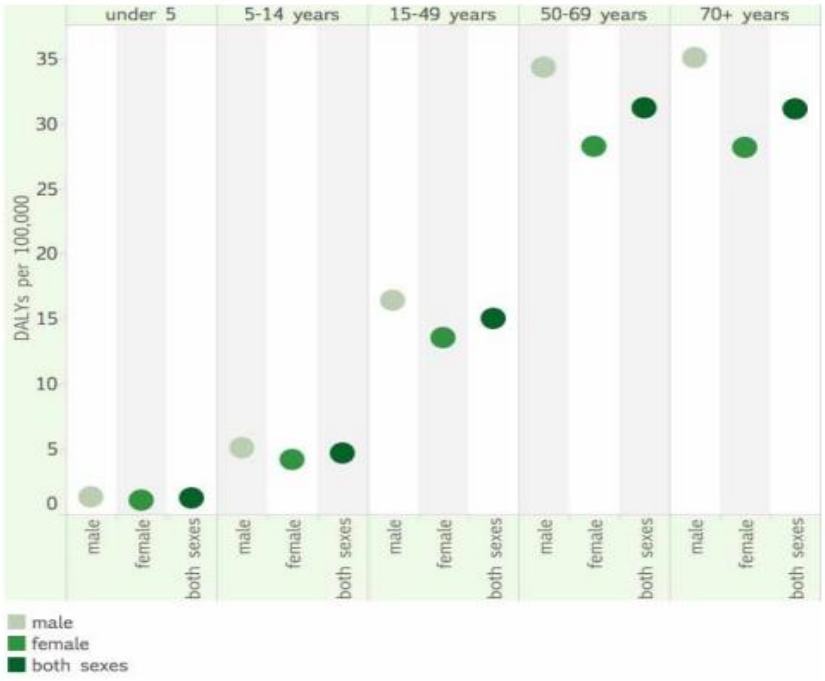

Figure 2. Distribution of DALYs for psoriasis per 100.,000., by gender and age group (2).

1.2. Clinical Effects

Psoriasis is a papulosquamous disease with variable morphology, Distribution, severity, and course. It is characterized by scaling papules (raised lesions are less than 1 cm in diameter), and plaques (raised lesions are greater than 1 cm in diameter).

People with psoriasis experience a range of associated comorbidities., including obesity, cardiovascular disease, non-alcoholic fatty liver disease, diabetes, and metabolic syndrome, at higher rates than the general population., particularly in those with more severe psoriasis (13). This results in a two to three-times higher mortality rate from cardiovascular disease compared to the general population (14).

Psoriasis is also strongly related to mental health issues., such as anxiety and depression, as it is a very visible disease to others and this causes feelings of self-stigmatization and major changes in style of life caused by psoriasis. Society's response to skin diseases is not one of understanding and empathy., but of disinterest and prejudice, since negative connotations are attributed to skin manifestations (14).

The impact of psoriasis on patients leads to a significant reduction in quality of life, comparable to that experienced in diseases such as cancer, diabetes, or heart disease. The decrease in quality of life affects physical, social, and psychological well-being.

The symptoms related to the skin, issues with the joints from psoriatic arthritis, and the challenges of living with an evident and disfiguring condition about difficulties in employment and social interactions, ultimately impacting overall mortality.

Given the substantial psychological impact of psoriasis., dermatologists must spend adequate time with each patient to ensure they feel supported and well-informed. Proper explanations about the disease, its triggers, available treatments, and their appropriate use can enhance treatment outcomes (15).

1.3. Clinical Presentation of Psoriasis

Psoriasis may also develop at the site of trauma or injury, a response known as the Köbner phenomenon. If psoriasis progresses or remains uncontrolled, it can lead to generalized exfoliative erythroderma and may involve the nails, particularly in the presence of psoriatic arthritis (PSA). Occasionally, psoriasis

can affect the mucosa or the lining of the mouth. When the tongue is involved, the dorsal surface may display swirling red spots with yellow-white borders. These lesions can evolve, expand, and change daily, sometimes assuming distinct cyclic patterns, resembling a map., which is referred to as geographic tongue. The morphology and distribution of psoriasis can be highly variable *(16)*.

Clinical manifestations of psoriasis appear in different ways: plaque., flexure., intestinal., pustular, or erythrodermic psoriasis. The most common form of psoriasis is plaque psoriasis, which presents as well-defined salmon-pink plaques with silvery-white scales that usually appear in symmetrical distribution and affect extensor surfaces (especially elbows and knees), trunk, and scalp (Figure 3). Spots of bleeding may be noted where scales have been removed (Ospitz's sign). In rare cases of severe, uncontrolled disease, psoriasis causes widespread erythematous rashes (erythroderma) that are life-threatening due to potential complications including hypothermia, risk of infection, and acute kidney injury.

Erythematous plaque lesions are covered with pearly white scales with well-defined borders that are characteristic and symmetrical patterns. They produce itching, pain, and bleeding. It mainly affects the extensor areas of the limbs., the lumbosacral region., and the scalp. Also, it can affect the genitals, the internal area of the gluteus, the umbilical area, and the joints (17).

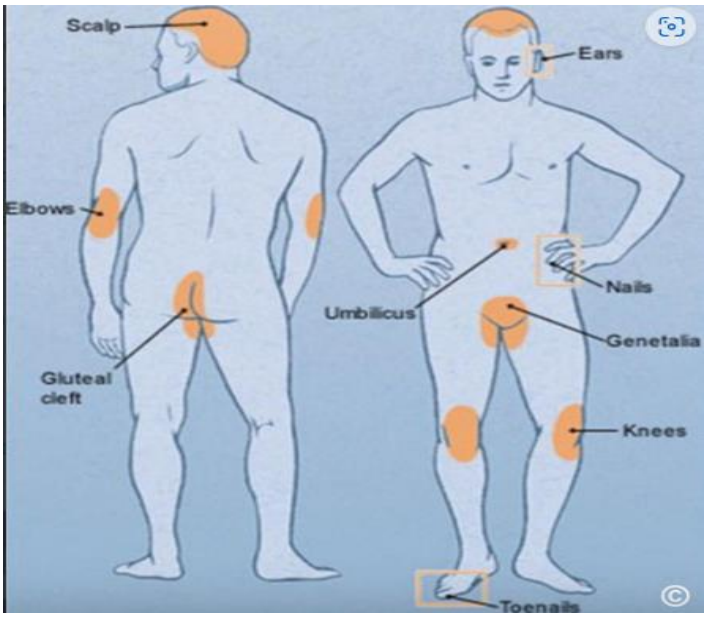

Figure 3. Distribution of psoriasis lesions (17).

Psoriasis manifests in various forms: plaque, guttate, flexural or intertriginous, pustular psoriasis, erythrodermic, and nail psoriasis *(13,14).* The lesions can itch and when the adherent psoriatic scales are scratched or removed, it can produce spot bleeding, known as Au spitz's sign (13) (Figure 4).

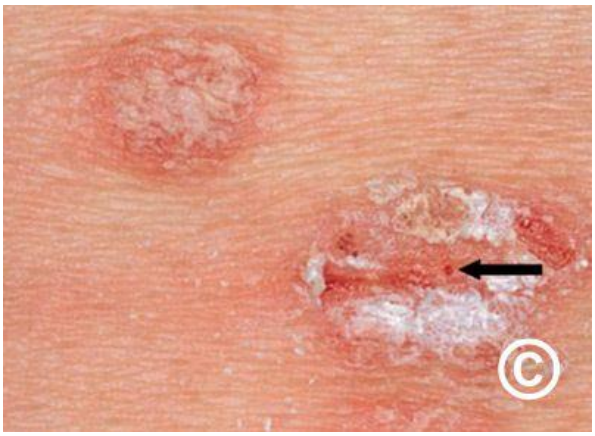

Figure 4. Sign of Auspitz. Scale removed to show pinpoint areas of bleeding (arrow) (18).

Psoriasis is a chronic immune-mediated inflammatory skin disease with phenotypically distinct subtypes such as plaque, flexural, intestinal, pustular, or erythrodermic.

The clinical presentation of psoriasis is classified as plaque psoriasis., guttate psoriasis, flexural psoriasis., generalized pustular psoriasis., erythroderma psoriasis., palmoplantar pustulosis psoriasis., and psoriatic nail disease.

1.3.1. Plaque Psoriasis

The most common form is plaque psoriasis., characterized by well-defined plaques in a symmetrical distribution and affects the extensor surfaces (especially the elbows and knees), the trunk, and the scalp (13).

Patients may present with sharply circumscribed, round-oval, or nummular (coin-sized) plaques. These lesions often start as erythematous macules (flat and <1 cm) or papules that extend peripherally and coalesce into plaques ranging from one to several centimeters in diameter. A white-whitish ring, known as a Woronoff ring., may be seen on the skin around the psoriatic plaque. With gradual peripheral extension, plaques may develop various configurations, including:

- Psoriasis gyrates Curved linear patterns predominate.
- Annular psoriasis: Ring-like lesions develop secondary to central clearing.

- Psoriasis follicularis: Minute scaly papules are present at the openings of pilosebaceous follicles.

Distinct morphological subtypes of plaque psoriasis include rupioid plaques, which are small (2–5 cm in diameter) and characterized by high hyperkeratosis, resembling limpet shells. Outrageous psoriasis: Hyperkeratotic plaques with relatively concave centers, similar in shape to oyster shells (Figure 5).

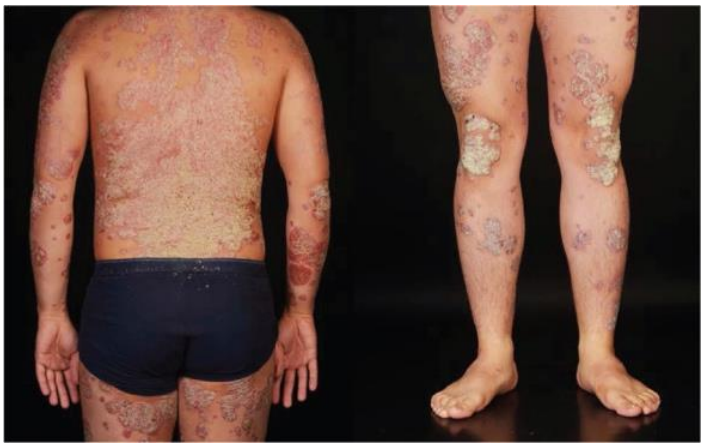

Figure 5. Plaque psoriasis. Generalized erythematous plaques., distributed symmetrically on the limbs and back., are well-defined (13).

Psoriasis usually has scales that are typically silvery white and can vary in thickness. Removal of the crust may show tiny bleeding points (Auspitz's sign). In acute inflammatory or exanthema-tic psoriasis, scaling can be minimal, with erythema as the predominant clinical sign *(13).*

1.3.2. Guttate Psoriasis

Guttate psoriasis, from the Latin word "gutta" meaning a droplet, describes the acute onset of myriad small (2–10 mm in diameter) lesions. These are usually distributed in a centripetal fashion., though they can also involve the head and limbs. Classically., guttate psoriasis occurs shortly after an acute group B hemolytic streptococcal infection of the pharynx or tonsils and can be the initial presentation of psoriasis in children or, occasionally, adults. The number of lesions may range from five or ten to over 100. Intestinal psoriasis accounts for 2% of all psoriasis cases. Gut psoriasis is characterized by multiple small "teardrop" lesions that typically affect most of the body. The rash appears very quickly and may follow a streptococcal infection. It usually affects children and young adults and has a high chance of clearing up spontaneously (18).

In children, an acute episode of guttate psoriasis is usually self-limiting, while in adults, guttate flares may complicate chronic plaque disease. Although few studies have assessed the long-term prognosis of children with acute guttate psoriasis *(19).* However, these patients may later develop plaque psoriasis (13). one small study revealed that 33% of these patients eventually developed chronic plaque disease *(20) (Figure 6).*

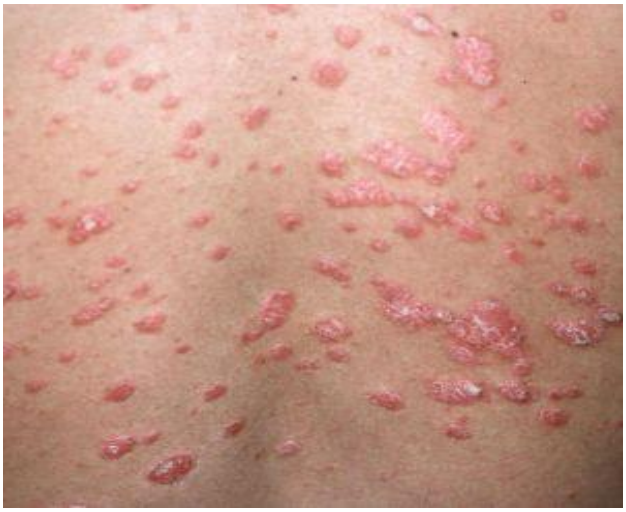

Figure 6. Back of a patient affected by gouty psoriasis (18).

1.3.3. Flexural (Inverse) Psoriasis

Flexural or intertriginous psoriasis affects areas of the body with folds such as the particularly inframammary, axillary areas, perineal, under the breasts, or the genitals (13) (Figure 7).

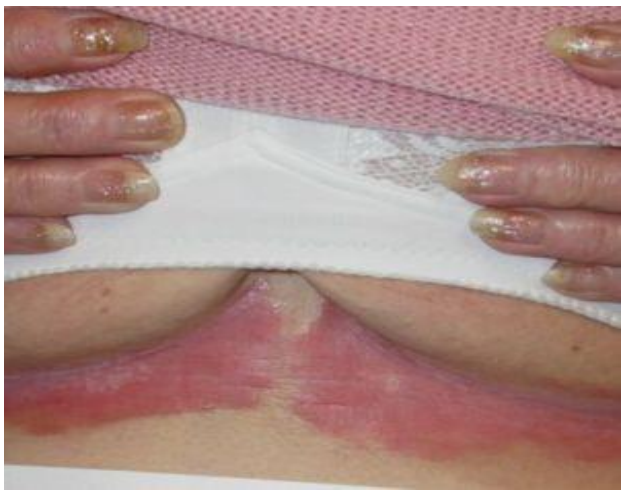

Figure 7. Affection of injuries in the sub-mammary area (18).

They are morphologically distinct from traditional plaques on the trunk and limbs. Non-scaly flexural lesions appear as red, shiny, well-defined plaques that are sometimes confused with candidiasis, intertrigo, and dermatophyte infections *(13).*

1.3.4. Generalized Pustular Psoriasis

Generalized pustular psoriasis (von Zumbusch) is rare and represents an active, unstable disease. which presents as generalized pustules on a background of red skin (18). Precipitating factors include the withdrawal of systemic or potent topical corticosteroids and infections. Patients present with pyrexia, red, painful, inflamed skin studded with monomorphic, sterile pustules that may coalesce to form sheets. Patients with generalized pustular psoriasis frequently require hospitalization for management *(13)* (Figure 8).

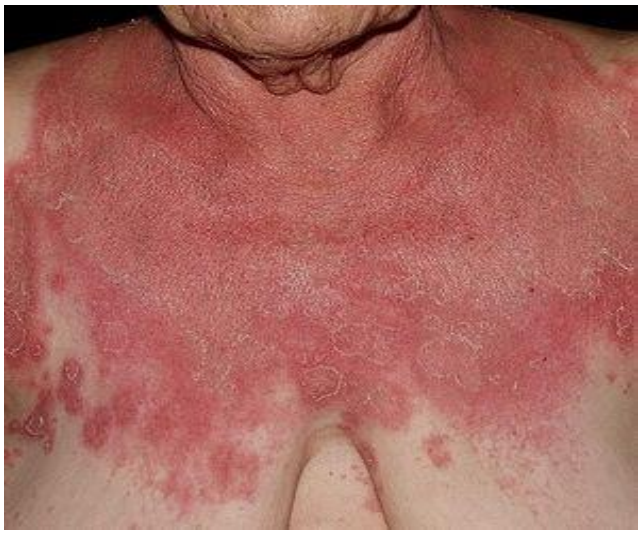

Figure 8. Patient with generalized pustular psoriasis in the chest and neck (18).

There is another type of pustular psoriasis., called palmoplantar pustulosis., which causes red pustules on the hands and feet (18).

1.3.5. *Erythroderma Psoriasis*

Erythrodermic psoriasis affects 90% of the patient's skin surface with an acute or subacute onset of generalized erythema with little desquamation *(21).*

Total or subtotal involvement of the skin by active psoriasis is known as erythroderma. This condition can manifest in two ways: In chronic plaque psoriasis, there is a possibility of gradual progression as the plaques become contiguous and spread. Erythroderma may be a manifestation of unstable psoriasis precipitated by infection, tar, drugs, or corticosteroid withdrawal. Erythroderma can impair the skin's thermoregulatory capacity., leading to hypothermia, high-output cardiac failure, and metabolic changes including hypoalbuminemia, hypothermia, electrolyte imbalances, and heart failure, and anemia due to the loss of iron, vitamin B12, and folate (13,21) (Figure 9).

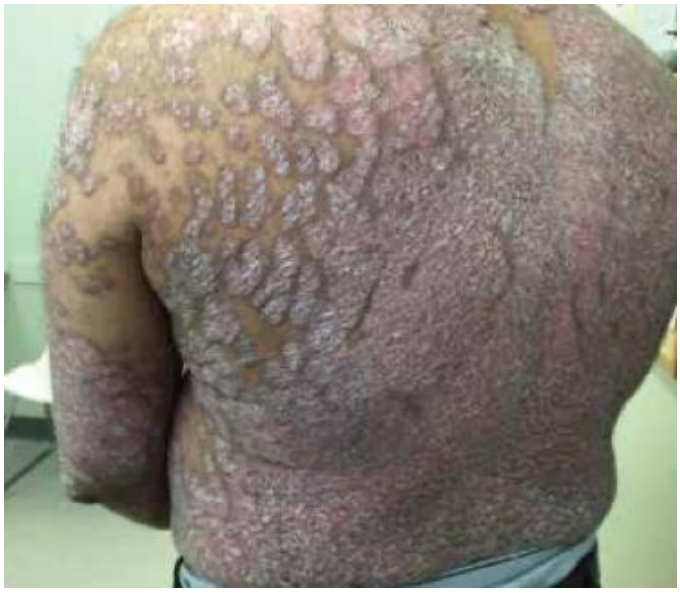

Figure 9. Patient with erythrodermic psoriasis affecting almost the entire skin surface of the back (21).

1.3.6. Palmoplantar Pustulosis Psoriasis

Palmoplantar pustulosis This type of psoriasis appears sterile, with yellow pustules on the background of erythema and scaling that affects the palms and/or soles of the feet. The pustules are tender and typically fade, leaving a dark brown coloration with adherent scale or crust. This condition is frequently associated with psoriatic nail involvement. Approximately 25% of cases are associated with classic psoriasis vulgaris., however, it is now believed that palmoplantar pustulosis may not be a form of psoriasis. This conclusion is based on genetic studies that show no association with HLA-Cw6 or other markers on chromosome 6p, which are linked to chronic plaque and guttate psoriasis.

The demographics of palmoplantar pustulosis differ markedly from those of chronic plaque psoriasis. Palmoplantar pustulosis more commonly affects women (9:1 ratio), typically presenting between the ages of 40 and 60 years. There is also a striking association with smoking., either current or past, in up to 95% of subjects *(22)* (Figure 10).

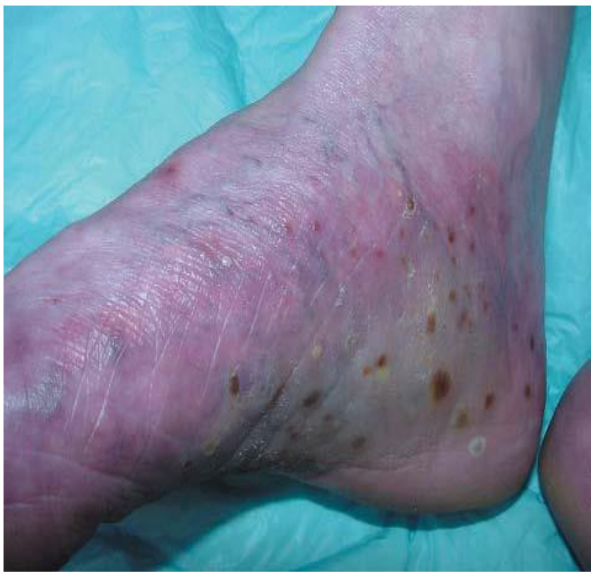

Figure 10. Patient with generalized Palmoplantar pustulosis. psoriasis in the chest and neck area (19).

1.3.7. Psoriatic Nail Disease

Nail psoriasis primarily occurs in patients who also have skin psoriasis., with less than 5% of patients only suffering from nail psoriasis. It presents with itching., distal onycholysis (separation of the nail bed)., subungual hyperkeratosis., oil drop sign (discoloration of the nail in one area)., splinter hemorrhages., leukonychia and sunken red lunula. Nail involvement is a predictor of psoriatic arthritis (14,18,21).

This kind of psoriasis more commonly affects fingernails than toenails. The most common finding is small pits in the nail plate., resulting from defective nail formation in the proximal portion of the nail matrix (Figure 11). The nail may detach from the bed at its distal or lateral attachments, a condition known as onycholysis (Figure 11). Orange-yellow areas., termed "oil spots.," may appear beneath the nail plate. The nail plate may also become thickened., dystrophic, and discolored (Figure 11). Yellow, keratinous material may collect under the nail plate., known as subungual hyperkeratosis *(19).*

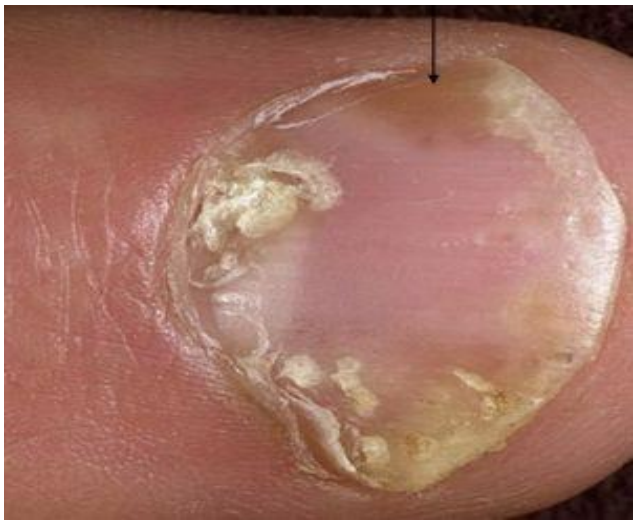

Figure 11. Psoriasis on the nail with the manifestation of an oil drop sign (18).

Sometimes., psoriasis develops in a combination of different types simultaneously, sequentially, or over time in the same patient (14). In addition to affecting the skin psoriasis can be associated with an inflammatory arthritis

known as psoriatic arthritis., which impacts the joints of the spine and other joints (2) (Figure 12).

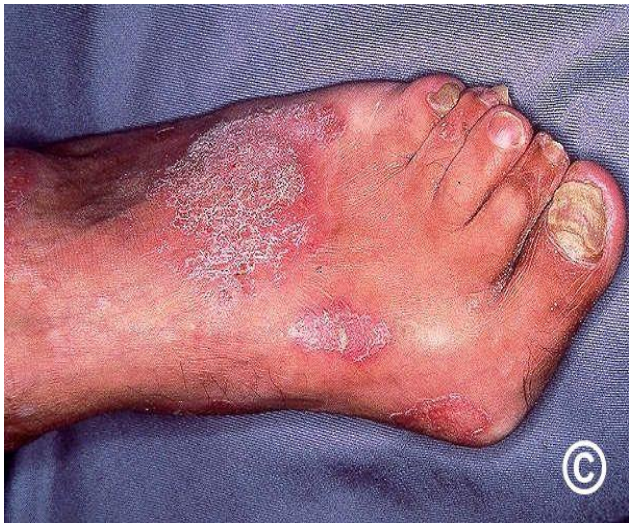

Figure 12. Psoriatic arthritis (18).

1.4. Aetiology

Psoriasis is a chronic autoimmune disease affecting the skin and joints. Clinical features include erythematous plaques covered by scales, with a recurrent, and chronic course. This is due to a hyperproliferation of the epidermis and blood vessels due to abnormal differentiation of keratinocytes, the infiltration of T lymphocytes, and various endothelial vascular changes of the epidermal layer *(23-24).* It has been seen that T lymphocytes and macrophages appear in the dermal infiltrates of psoriasis lesions before significant epidermal changes develop *(24).*

The involvement of T lymphocytes in the pathogenesis of psoriasis occurs in three steps: the initial activation of T lymphocytes., migration to the skin, and the release of cytokines by T lymphocytes *(24).*

Activation: requires at least 2 signals from the antigen-presenting cell (APC): the first signal occurs when the major histocompatibility complex (MHC) presents an antigen to a T cell receptor, and the second by a co-stimulation that occurs when lymphocyte function-associated antigen 3 (LFA3) stimulates CD2, B7 stimulates CD28, and an intracellular adhesion molecule (LFA1) stimulates the resting T cell surface (Figure 13) *(24).*

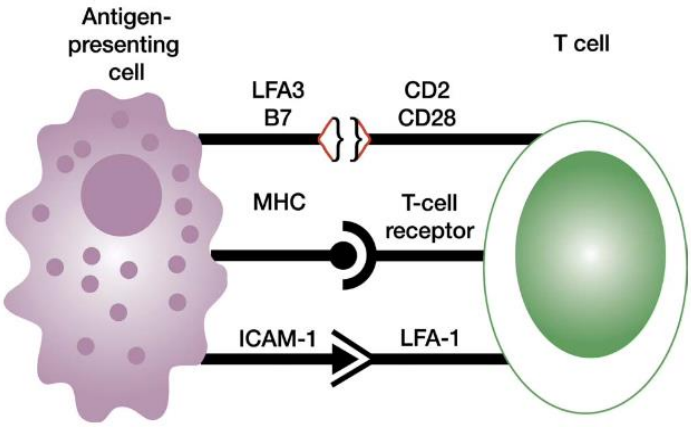

Figure 13. T cell activation process by the action of the antigen-presenting cell (24).

- Migration: Activated T lymphocytes expand, causing a proliferation of T lymphocytes that recognize antigens. Lymphocytes access the circulatory system and through cell-cell interactions with the endothelial cells of the blood vessel, migrate to the inflamed skin *(24).*
- Function of cytokines: once the skin is inflamed., lymphocytes encounter the initiator antigen, and release a series of cytokines., which play a central role in the phenotypic expression of psoriasis, such as interferon (INF γ) (which inhibits keratinocyte apoptosis), interleukins-2 (IL-2) (which stimulate the growth of T lymphocytes) and tumor necrosis factor (TNF) (which increases the proliferation of keratinocytes, of cytokines

pro-inflammatory cells, T lymphocytes, macrophages, and vascular endothelial cell adhesion molecules) *(24).*

- Genetic Component in Psoriasis: There is a genetic component in the development of this disorder., as different loci in the genome have been identified as linked to psoriasis susceptibility. The most studied locus is PSORS1, this locus is located within the MHC region of chromosome 6, contains the genes that code for proteins related to immune function and is strongly linked to genes for human lymphocyte antigens in this region. It has also been observed that there are genetic connections between psoriasis, and other diseases, such as atopic dermatitis, rheumatoid arthritis., or Crohn's disease, this is because these diseases have a series of common pathological links such as the overproduction of TNF and INFγ (14,24-25).
- Immune Activation in Psoriasis: The complex interaction between T cells, dendritic cells, and keratinocytes is the likely driver of immune activation, chronic inflammation, and proliferation of keratinocytes (13). Hyperplasia and altered differentiation of keratinocytes are classic characteristics of psoriatic lesions. In psoriasis, the population of proliferative cells doubles, the cell cycle being 8 times shorter and lasting 36 hours per day, as opposed to the normal skin where its cycle lasts 311 hours. The production of keratinocytes in psoriasis lesions is approximately 28 times greater than in a normal epidermis (24).

Considering the 60 million people worldwide who are suffering from this disease, its specific prevalence varies between countries. Therefore, psoriasis's pathogenesis is multifactorial, with genetics being the main factor, especially in people with early-onset plaque psoriasis (less than 40

years of age). This was demonstrated by twin, family-based, and large-scale population-level studies, with heritability estimated at 60-90%. More than 60 susceptibility loci have been identified using genome-wide association studies *(26).* Many of the candidate causal genes are involved in antigen presentation (HLA-C and ERAP1)., NF-kappa B signaling (TNIP1), type 1 interferon pathway (RNF113 and IFIH1), interleukin (IL)-23/Th17 axis (IL23R, IL12B, and TYK2) and skin barrier function (LCE3) *(26).* This suggests a complex interplay between T cells, dendritic cells, and keratinocytes as the likely underlying pathophysiology of psoriasis, with the IL-23/Th17 axis being the central driver of immune activation, chronic inflammation, and keratinocyte proliferation *(27).* Environmental triggers have been known to exacerbate psoriasis such as obesity, stress, beta-blockers, smoking, and lithium *(28).* Although there is a relative paucity of data, pustular psoriasis appears to be genetically distinct, with different susceptibility genes implicated (IL36RN, AP1S3 in those of European descent, and CARD14) *(26,29).*

1.5. Molecular genetic basis of psoriasis

The molecular genetic basis of psoriasis is complex., involving multiple genes. Seven main sites of susceptibility to psoriasis have been reported. The heritability of psoriasis is estimated between 60 and 90%., which has a main genetic component. Many researchers have identified the primary susceptibility locus at 6p21, known as PSORS1., which is overrepresented in all tested populations *(30-31).* Other susceptibility sites have also been

identified on chromosome 1p (PSORS7) *(32).* 1q (PSORS4) *(33).* 3q (PSORS5) *(34).* 4q (PSORS3) *(35).* 17q (PSORS2) *(36),* and 19p (PSORS6) *(37).* However, the replication of these findings has been incomplete, suggesting variability in psoriasis susceptibility beyond PSORS1. This variability may be partly due to genetic heterogeneity among different populations. While the genetic component of psoriasis is well-established, the precise locations of the involved genes remain to be definitively determined.

1.6. Diagnosis

This disease is diagnosed based on clinical findings such as skin rash, changes in the nails, or joint involvement, although it is usually a simple diagnosis, skin lesions must be well differentiated from other diseases such as ringworm, mycosis, or seborrheic dermatitis (14).

To quantify the severity of the disease, several clinical measures are used:

- Body Surface Area (BSA): this consists of a direct calculation of the affected body surface, calculated using the palm of the patient's hand as 1% of the body surface (the maximum score is 100%, with involvement greater than 10% is considered severe psoriasis (38).
- Psoriasis Area and Severity Index (PASI): This is the standard for measuring the severity of psoriasis. It evaluates the degree of erythema, desquamation, and infiltration of psoriasis plaques with a

compromised topographic relationship. The score ranges from 0 to 72, with more than 10 points indicating a severe condition (38).

- Dermatology Life Quality Index (DLQI): This is a 10-question questionnaire for the patient, covering symptoms., sensations, daily activities, leisure., work or school., personal relationships, and treatment. The score ranges from 0 to 30, with values higher than 10 considered severe psoriasis (38).

1.7. Treatment

The history of psoriasis has been a long journey since ancient times this disease was confused with leprosy and other skin disorders. The identification of psoriasis did not occur until the 19th century when they were finally able to distinguish it from other skin pathologies *(25,39-40).*

Significant advancements in understanding this common, disfiguring, and socially stigmatized skin disease have been made over the years. Psoriasis can cause significant discomfort and impact quality of life. While there is no cure for psoriasis, various treatments aim to manage symptoms and improve skin appearance. Growing knowledge about the disease has facilitated the detailed targeting of specific immunological processes.

Newly targeted therapies are shedding light on the pathophysiology and treatment of psoriasis, as well as other immune-mediated diseases *(39).*

These treatments include topical therapies and systemic medications. Treatment choice depends on the severity of the condition and individual

patient response, often requiring a combination of approaches to achieve optimal results.

Over the past two centuries, most therapies have been developed empirically, and countless drugs have been employed to treat psoriasis *(41).* Advances in understanding its pathophysiology have led to the development of highly effective and targeted treatments *(13).* Currently., in the therapy of psoriasis we can find several modalities, to choose one of the treatments., the specific factors of the patient., their preferences, and adherence must be taken into account. Treatment targets include at least 75% or 90% improvement in PASI (PASI75 or PASI90), translating to absolute PASI scores of 4 or 2, respectively (42).

Psoriasis can manifest in various high-impact and difficult-to-treat sites, including the scalp, face, nails, genitalia, palms, and soles. These sites often require specialized treatment approaches due to their sensitivity and the significant discomfort they can cause. Effective management of psoriasis extends beyond addressing skin symptoms, it also involves recognizing and treating associated comorbidities (such as psoriatic arthritis, and psychological, cardiovascular, and hepatic diseases. A comprehensive, holistic approach is essential to improve the overall well-being, and quality of life for individuals with psoriasis. Therapeutic options for this disease include conventional systemic agents such as methotrexate, cyclosporine, and acitretin). Phototherapy methods involve narrow-band ultraviolet B (NB-UVB), psoralen, and ultraviolet A (PUVA) are also utilized. Additionally., biological treatments targeting (TNF), interleukin (IL)-17 and IL-23 inhibitors, and small molecule inhibitors such as dimethyl fumarate and apremilast, offer advanced options for managing the condition. Topical therapies., including

corticosteroids, and vitamin D analogs, are fundamental in treating localized psoriasis symptoms (13).

1.7.1. Systemic Therapy

The systemic therapy can be divided into non-biological treatments (Table 2) and biological treatments (Table 3).

Table2. Conventional non-biological treatments (23,38).

Methotrexate	It is a synthetic folic acid analog exhibiting immunosuppressive and anti-inflammatory properties.
Ciclosporin A	It is a powerful immunosuppressant., and calcineurin inhibitor that blocks pro-inflammatory signals.
Apremilast	It is an inhibitor of phosphodiesterase 4., which causes an increase in the intracellular level of cAMP, which causes the inhibition of the inflammatory response.
Acitretin	It is the active metabolite derived from etretinate., modulates epidermal proliferation and differentiation, and also has anti-inflammatory and immunomodulatory effects.
Phototherapy	It is UV radiation in its wavelengths., UVA (320-400nm) penetrates the dermis., alters the expression of cytokines., and causes immunosuppression and lymphocyte apoptosis.

1.7.1.1. Methotrexate and Acitretin

Methotrexate works by inhibiting lymphocytes via multiple mechanisms including dihydrofolate reductase inhibition., aminoimidazole carboxamide ribotide transformylase (AICARTase) blockade, and adenosine accumulation. Its most important complication is bone marrow suppression.

Other potential complications of treatment include nausea, pneumonitis, hepatitis, liver fibrosis, and teratogenicity. Methotrexate is usually taken orally every week. Subcutaneous formulation causes fewer gastrointestinal side effects and is more efficacious due to higher bioavailability Ciclosporin is a calcineurin inhibitor (43), and has a rapid onset of action but may cause hypertension and irreversible renal toxicity.

Eating Acitretin., which is a retinoid, causes keratinocytes to differentiate. Its possible side effects include dry skin, hair loss, hyperlipidemia, and hepatotoxicity.

Methotrexate and acitretin are contraindicated in pregnancy for disease (44), refractory to methotrexate and/or ciclosporin, or where second-line therapies are unsuitable, biological therapies or oral small molecule inhibitors may be considered.

1.7.1.2. Cyclosporine and Apremilast

Cyclosporine mediates its anti-psoriasis effects through selective inhibition of immunological pathways in T cells (45). This immunosuppressive drug is ineffective or inappropriate for the treatment of severe psoriasis when conventional therapy is available (46-47). It is recommended for use in short-

term treatment (45,48). Long-term cyclosporine treatment is not recommended due to AEs (in particular., nephropathy and hypertension), and risks of end-organ toxicity (49). In addition., cyclosporine increases the risks of non-melanoma skin cancer (NMSC), and carcinogenic potential during long-term treatment [(1-2 years)] (50).

Apremilast, an orally administered small molecule phosphodiesterase 4 (PDE4) inhibitor., was approved by the U.S. Food and Drug Administration in 2014 for the treatment of active psoriatic arthritis, and moderate-to-severe plaque psoriasis (51).

The efficacy and safety of apremilast have been well established through clinical trials, demonstrating superior outcomes compared to placebo. Apremilast has also shown effectiveness in improving psoriasis in challenging areas, such as the palms, nails, and scalp, while significantly reducing itching and enhancing patient quality of life. However, in head-to-head comparisons from pooled trial data, apremilast was found to be less effective than calcipotriol/betamethasone dipropionate (52) .

Long-term clinical trials of apremilast, with up to 3 years of follow-up, have identified the most common side effects. These include gastrointestinal issues such as nausea and diarrhea, infections such as upper respiratory tract infections, and nasopharyngitis, as well as headaches (53-55).

1.7.1.3. Phototherapy

Phototherapy is considered a second-line treatment for moderate to severe psoriasis, often used when topical treatments are insufficient. This approach includes (NB-UVB) and psoralen ultraviolet A (PUVA) therapies. NB-UVB has become the preferred method over PUVA due to the latter's increased risk of skin cancer with cumulative PUVA exposure (56). Alongside phototherapy, systemic agents such as methotrexate, cyclosporine, and acitretin are also employed to manage more severe cases of psoriasis. These treatments aim to reduce inflammation and slow down the rapid skin cell turnover characteristic of the condition.

1.7.1.4 Biological Treatment

Traditional non-biologic psoriasis therapies can negatively impact the quality of life due to various factors, including discomfort and toxicity. In response, biological agents targeting the immuno-pathogenesis of psoriasis have been developed. Some of these agents are now approved for clinical use based on favorable efficacy, and safety results in clinical trials (57).

The introduction of biologicals represented a milestone in the treatment of moderate-to-severe psoriasis. Biological treatments are listed in (Table 3). The tumor necrosis factor (TNF) antagonists etanercept, adalimumab, and infliximab, as well as the interleukin 12/23 antagonist ustekinumab, were available as second-line therapies for refractory psoriasis, and psoriatic arthritis until the beginning of 2015 (58).

Table 3. Characteristics of biological therapies according to Catsalut (59).

NAME	*THERAPEUTIC TARGET*	*STRUCTURE*	*INDICATION*
Adalimumab	TNFα	Monoclonal antibody (humanized IgG)	1ª LINE Moderate to severe plaque psoriasis in adults' candidates for systemic therapy
Secukinumab	IL-17A	Monoclonal antibody (fully human IgG1/x)	
Ixechizumab	IL-17A	Monoclonal antibody (humanized IgG4)	
Etanercept	TNFα	TNF protein p75 fused to the Fc of a human IgG1	2ª LINE Moderate to severe plaque psoriasis in adult patients who do not respond to., have a contraindication to, or are intolerant to cyclosporine., methotrexate., or PUVA therapy
Infliximab	TNFα	Monoclonal antibody (chimeric IgG).	
Ustekinumab	IL-12/IL-23	Monoclonal antibody (fully human IgG1)	

All agents used in the treatment of psoriasis are highly effective and have a favorable risk-benefit profile (60-62), significantly contributing to health-related quality-of-life improvement (63-65). Satisfaction with biological treatments is notably higher compared to others (63,64,66-68), due to their superior efficacy, and the convenience of their administration. Indeed, patient satisfaction largely depends on the process attributes of the

treatments prescribed, e.g., treatment location and time required for administration (66,69). Conversely, a treatment process incompatible with an individual's preferences or personal, and professional needs is likely to result in dissatisfaction, and nonadherence, problems commonly encountered in psoriasis. Hence, greater integration of patients' preferences into shared decision-making is warranted to optimize treatment outcomes.

Multiple biological therapies are approved for use in moderate-severe psoriases such as TNF (adalimumab, etanercept, infliximab, and certolizumab), IL-12/23p40 (ustekinumab), IL-23p19 (risankizumab, guselkumab, and tildrakizumab), IL-17 (ixekizumab and secukinumab), and IL-17 receptor (brodalumab) inhibitors.

Currently, this is primarily influenced by clinical (70-71). information eg psoriasis factors (disease phenotype, presence of PsA, and outcomes of previous biologic treatment), comorbidities (demyelinating disease, and inflammatory bowel disease), drug-specific factors (dosing frequency), and lifestyle considerations (conception plans) (70). Genomic information has the potential to guide the effective deployment of therapies in the future (72).

Although biological drugs are very effective, they require regular subcutaneous or intravenous administration. Oral small-molecule co-inhibitors including apremilast (a phosphodiesterase 4 inhibitor), and dimethyl fumarate are approved for use in moderate to severe psoriasis, and trials for this molecule tyrosine kinase 2 blockers in the Janus kinase (JAK)-activated protein transducer, and activator of transcription proteins (STAT) pathway (13).

Evidence suggests that patients receiving biological therapies experience significant improvements in health-related quality of life (57).

In a study evaluating the four-year drug survival of TNF antagonists, maintenance rates ranged from 70 % for infliximab to 40 % for Etanercept or Adalimumab (73). Levine and colleagues identified Infliximab as the biological with the longest sustainability, and ustekinumab as the biological with the lowest failure rate (74).

This data is at odds with findings published by Brunasso et al. who showed a lower four-year drug survival rate for infliximab than for etanercept, and adalimumab (75).

1.7.2 Topical Therapy

The earliest attempts at treating psoriatic lesions topically date back to antiquity, but the effective development of these treatments did not begin until the 19th century (40).

It is the therapy most used in mild to moderate psoriasis. For treatment., 90% of the patients are treated using topical formulations., due to their affordable, safe, and convenient administration. Besides classical anti-psoriatic drugs including topical corticosteroids, vitamin D analogs, tar-based preparations, and active agents in traditional medicines, nanotechnology is also utilized to improve drug penetration, and bioavailability by enhancing drug affinity to skin barriers or by improving the physicochemical properties of the drug for

topical delivery (Table 4). presents the topical treatments available for managing psoriasis.

Table 4. Topical approaches available for psoriasis treatment (23).

Corticosteroids	Betometasone dipropionate., Clobetasol propionate., Mometasone., Fluticasone propionate., Methylprenisolone aceponate.
Vitamin D analogs	Calcitriol., Calcipotriene., Tacalcitol., Maxacalcitol
Retinoids	Tazarote
Calcineurin inhibitors	Pimecrolimus., Tacrolimus
Antimitotic	Anthraline
Moisturizers and keratolytic	Salicylic acid., Urea

The efficacy of topical treatment can be increased with occlusion or combination therapy (e.g. calcipotriol/betamethasone).

Dithranol and tar, which used to be the answer to treatment, are less commonly used because they stain, and irritate the skin.

Psoriasis at difficult-to-treat sites (scalp, face, nails, genitalia, palms, and soles) warrants special attention due to its profound impact on function, and relatively poor response to treatment (Figure 14). Steroids used for face or genitalia should be of low potency and limited to short-term use due to the risk of skin atrophy, and telangiectasia.

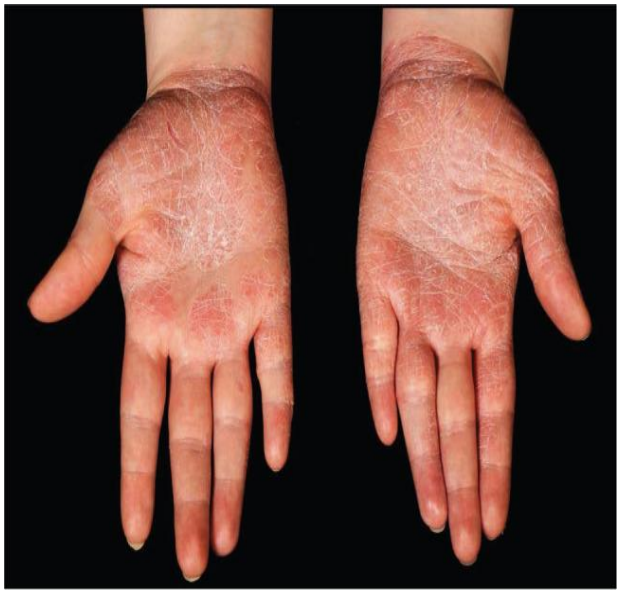

Figure 14. Palm psoriasis. When psoriasis affects areas such as the palms., soles., scalp., face., nails., or genitals., the treatment becomes more complicated and significantly affects the daily routine (13).

Biologics that target pro-inflammatory cytokines are monoclonal antibodies or soluble receptors. They have had a significant impact on outcomes in moderate to severe disease.

A novel topical treatment named taping of (GSK2894512, DMVT-505, VTAMA™) (approved by the FDA in May 2022), has been developed for the treatment of plaque psoriasis in adults. Also, Research has investigated the clinical utility of taping of (i.e., GSK289451, DMVT-505), a novel, first-in-class, topically administered, small-molecule modulator of the aryl hydrocarbon receptor in the treatment of psoriasis (76).

1.7.2.1. Corticosteroids

The treatment landscape for psoriasis has seen significant advancements, particularly in using biological agents for moderate-to-severe disease. However, due to factors such as limited disease area or patient preference, biological agents may not always be the treatment of choice. Instead, topical medications, like corticosteroids, remain the most commonly used therapies (77). However, limitations to the consistent use of these therapies exist. For example., the use of high-potency steroids needs to be avoided in areas like the face, and intertriginous regions (78). Further., the potential for adverse effects with prolonged use (eg., steroid atrophy) limits the duration of use (79).

Topical corticosteroids are the first-line treatment for psoriasis due to their antiproliferative, and anthropogenic properties. They reduce the expression of inflammatory mediators such as prostaglandins, and leukotrienes, and inhibit cytokine production (23). The potency of the prescribed corticosteroids varies based on the severity of the psoriasis.

Three factors determine the pharmacokinetics and potency of a topical corticosteroid: its structure, the vehicle used, and the skin area to which it is applied. Hydrocortisone is the central structure of most topical corticosteroids. Modifying or adding functional groups to the hydrocortisone molecule alters its lipophilicity, solubility, percutaneous absorption, and ability to bind to glucocorticoid receptors. The potency of the molecule is chosen based on the severity and location of the skin disease. The vehicle is selected based on the type of lesion, the need for hydration, the site of application, potential irritation from vehicle components, and patient

preferences. Patients often prefer vehicles that are quickly absorbed, easy to apply, and non-greasy (80).

Bioavailability, and penetration of topical steroids increase when the skin is inflamed or diseased., as well as with increased hydration of the stratum corneum. The thickness of the stratum corneum is inversely proportional to the penetration of topical corticosteroids. This is why very occlusive agents like ointments enhance absorption by increasing the hydration of the stratum corneum (79).

They have also been shown., that topical corticosteroids are related to inflammation in the body, and affect both adaptive and innate immunity by decreasing the number, and function of Langerhans cells, reducing immune responses in the skin, and decreasing neutrophils and leukocytes, thus reducing their phagocytic function and cellular toxicity. They also lower the production of many cytokines and decrease the mitotic rate of the epidermis, leading to reduced thickness of the stratum corneum (79).

Topical corticosteroids can be combined with other therapies., such as vitamin D analogs, as there is a synergistic effect. Other combinations would be: with phototherapy, ciclosporin, tazarotene, or anthralin (79).

Although this is a very effective therapy, their effectiveness, particularly for treating outbreaks, and localized lesions., topical corticosteroids have adverse effects such as skin atrophy., striae., and purpura. Therefore., their use should be limited. Repeated application can lead to tachyphylaxis, a loss of clinical efficacy (79).

1.7.2.2. Vitamin D Analogues

Vitamin D analogues are commonly used in the treatment of psoriasis due to their ability to regulate skin cell production and reduce inflammation. Vitamin D analogues include Calcitriol, Calcipotriene, Tacalcitol, and Maxacalcitol. These synthetic compounds mimic the effects of natural vitamin D, slowing down the rapid growth of skin cells and promoting normal cell turnover. This helps to clear psoriatic plaques and reduce scaling. Topical formulations are often prescribed for mild to moderate psoriasis and can be used alone or in combination with other treatments like corticosteroids for enhanced efficacy there is also part of the first line of topical therapies vitamin D analogs 20 (calcipotriol).

Calcitriol, the active form of vitamin D3, has been observed to inhibit keratinocyte proliferation and modulate their differentiation (23). The vitamin has anti-inflammatory properties, increasing anti-inflammatory cytokines while decreasing pro-inflammatory ones. Additionally, Calcitriol inhibits the production of IL-2, and IL-6 by T cells, blocks the transcription of interferon, and inhibits cytotoxic cell activity. The primary side effects include burning, and irritation at the application site, which are self-limiting, and resolve quickly once treatment is discontinued (79).

For patients undergoing long-term treatment., monitoring serum parathyroid hormone, and vitamin D levels is essential. Those with underlying conditions are at increased risk of developing hypercalcemia and should be checked regularly (79).

Vitamin D analogs have demonstrated efficacy comparable to medium-potency corticosteroids, although they are less effective than high-potency corticosteroids for psoriasis treatment (79).

Therefore, they are often used in combination with corticosteroids to achieve a synergistic effect, improving clinical response rates and minimizing the side effects of both treatments (79).

1.7.2.3. Retinoids

Retinoids are a class of compounds derived from vitamin A that are widely used in the treatment of various skin conditions, including psoriasis. These agents work by modulating cellular growth, and differentiation, making them effective in reducing the symptoms of psoriasis. Retinoids help to normalize the rapid turnover of skin cells and reduce inflammation, which are key factors in managing this chronic condition. Among the topical retinoids, tazarotene stands out for its efficacy and specific mechanism of action. It is a synthetic topical retinoid and a prodrug of tazarotenic acid. It binds to nuclear retinoic acid receptors, resulting in decreased inflammation, normalization of keratinocyte hyperproliferation, and abnormal differentiation seen in psoriasis (23).

As a retinoid, tazarotene is teratogenic and contraindicated in pregnancy. It is also photocarcinogenic, necessitating the use of sun protection. Adverse effects include burning, itching, stinging, and erythema (79).

Although effective as monotherapy, tazarotene is commonly combined with topical corticosteroids to improve tolerance, enhance efficacy, and decrease the anthropogenic potential of corticosteroids (79).

1.7.2.4. Topical Calcineurin Inhibitors

Topical calcineurin inhibitors, such as pimecrolimus, and tacrolimus, are immunomodulators that inhibit calcineurin phosphate activation, and the production of inflammatory substances, thus reducing psoriasis lesions (23).

Pimecrolimus is a derivative of Ascomycin macrolactone, and Tacrolimus is isolated from the bacterial strain Streptomyces tsukubensis. These inhibitors are effective in treating intertriginous, facial, and genital psoriasis. A common adverse reaction is burning at the site of application of the drug (79).

1.7.2.5. Antimitotic

Antimitotic agents work by disrupting the mitotic process, thereby inhibiting the excessive growth of keratinocytes which is typical in psoriatic lesions. Among the various antimitotic treatments, anthralin is used for treating psoriasis. It inhibits DNA replication and repair synthesis, interferes with mitochondrial function, and reduces the expression of key growth factors involved in skin cell proliferation. Additionally, Anthralin has been shown to decrease the production of inflammatory cytokines, and leukotrienes, contributing to its therapeutic effects. Specifically, anthralin inhibits DNA

replication, and repair synthesis, interferes with mitochondrial function, and decreases keratinocyte transforming growth factor expression, and epidermal growth factor receptor binding. It also inhibits leukotriene production by neutrophils and the secretion of IL-6, IL-8, and TNF-α. However, its use can lead to adverse effects such as skin, nail, and clothing staining due to oxidation, burning, and irritant or allergic contact dermatitis (79).

1.7.2.6. Moisturizers and Keratolytics

These products reduce scaling, limit painful lesions, and provide antipruritic benefits, making them a basic therapy for psoriasis (79). Moisturizers help maintain skin hydration, reduce dryness, and improve the skin barrier function, which is often compromised in psoriasis. Keratolytics, such as salicylic acid, and urea, aid in the removal of scales, and dead skin cells by breaking down the keratin in the skin.

1.7.2.7. Topical Medication Carrier Systems

Topical therapy is essential for treating mild to moderate psoriasis. However, the efficacy and compliance of topical treatments remain significant concerns. Many patients with psoriasis report dissatisfaction or only moderate satisfaction with their current treatments. The primary reasons for patient non-compliance include ineffective drug delivery and undesirable skin interactions with topical treatments (80). New delivery systems balance the

physicochemical requirements for the stability of active, and inactive constituents, preservation against microbial deterioration, and effective presentation of active molecules to the skin in a system that will allow the appropriate release of the active to the skin. Making the active more available to the target tissue allows greater effectiveness by controlling the concentration, and distribution of these actives within the stratum corneum of the skin, and the benefits can be optimized. Therefore., some different colloidal systems are used during the topical delivery of drugs. Very useful formulations can be found for psoriasis (81) (Table 5).

Table 5. Various colloidal carriers are used during topical drug delivery (80-83).

Drug Carrier Systems	*Description*	*Size*
Liposomes	Vesicles are formed by a bilayer of phospholipid molecules and water enclosed in these bilayers.	40-300 nm
Niosomes	Vesicular carriers are composed of nonionic surfactants instead of phospholipids.	
Microemulsions	Thermodynamically stable., isotopically clear., and transparent vehicles composed of oil., aqueous phase., and surfactants. They are super solvents.	10-200 nm
Dendrimers	Branched., roughly spherical repeating large molecules for drug delivery.	

Ethosomes	Liposomal systems are formed by high alcohol content., flexible vesicles., and high drug loading.	
Microcapsules	The system is formed through an emulsion that creates an insoluble film around each particle.	50-500 nm
Transfersomes	They are highly flexible deformable vesicles capable of bypassing the stratum corneum in a non-invasive way. They are composed of several bilayers of phospholipids.	

These carrier systems offer storage opportunities for drug molecules within their inner micelles according to their steric, and physicochemical properties. The association of drugs with carriers is usually non-covalent, relying on the collective strength of weak binding forces (23) (Figure 15).

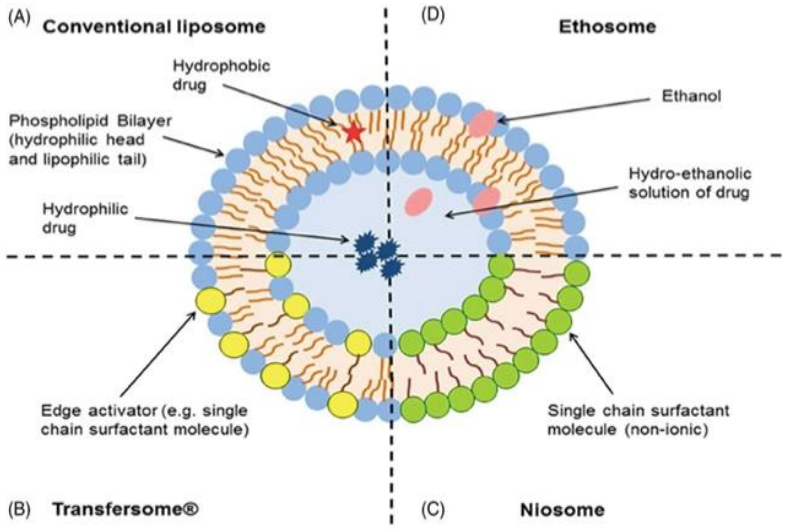

Figure 15. Structure of the different vesicular systems (84).

According to the pathogenesis of psoriasis and the current treatments, including systemic, and topical treatments, and the review of drug delivery systems with a special focus on their characteristics, the most effective topical drugs used for the treatment of psoriasis are always provided. But as it was said in the previous section., topical drugs are also associated with side effects, the main goal of this research was to create a new formulation for psoriasis treatment. To achieve this goal., we have presented a new formulation of baricitinib for the topical treatment of psoriasis to enhance the quality of life for those affected by psoriasis.

2. Baricitinib

Baricitinib (BCT) has the IUPAC name of 2-[1-Ethylsulfonyl-3-[4-(7H-pyrrolo[2,3-d] pyrimidin-4-yl) pyrazol-1-yl] azetidin-3-yl] acetonitrile *(85).* This drug has the chemical formula (C16H17N7O2S., formerly LY3009104) (OlumiantTM), and a molecular weight of 371.42 g/mol *(86).* Incyte and Eli Lily first developed this small molecule, a reversible Janus-associated kinase (JAK) inhibitor in more than 65 countries, including Japan. In February 2017, it received approval in the European Union as a second-line oral therapy for the treatment of adults with moderate to severe rheumatoid arthritis (RA) *(87-88).* Also, this drug has the advantage of providing antiviral activity in vitro at concentrations that are approved by the dose *(89).* It is used as a monotherapy or in combination with methotrexate *(90).* The chemical structure of Baricitinib (Figure 16) shows that the drug is basic and polar.

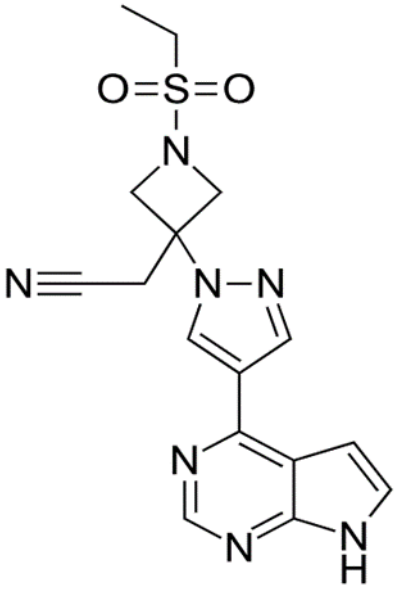

Figure 16. The chemical structure of Baricitinib (86).

2.1. Pharmacology and Pharmacodynamics

Baricitinib is a selective inhibitor of Janus kinase (JAK), belongs to the first generation of JAK1/2 inhibitors, and plays a crucial role in modulating the JAK-STAT signaling pathway, which is fundamental to the regulation of immune responses, and inflammation. It can penetrate inside the target cell membrane under its small molecular size and interaction with intracellular protein moieties *(91).*

Janus Kinase (JAK) family belongs to a subgroup of non-receptor protein tyrosine kinases which are linked to the intracellular domains of many cytokine receptors. They are characterized by two adjacent domains resembling the face of the Roman God Janus *(92).*

JAK kinases are intracellular enzymes involved in cytokine signaling., inflammation, immune function, and hematopoiesis, they are also upregulated and mutated in various tumor cell types. Four cytoplasmic protein tyrosine kinases make up the JAK family: JAK1., JAK2., JAK3., and tyrosine kinase 2 (TYK2) *(93).* Based on cytokines, these may homo- or hetero-dimerize and result in autophosphorylation. This in turn will phosphorylate and recruit STAT proteins (Signal Transducer and Activator of Transcription, 7 types: 1, 2, 3, 4, 5a, 5b, and 6), which will eventually result in the transcription of inflammatory mediators *(94-95).*

Basic, and translational science has identified a wide array of subcellular pathways that regulate normal, and aberrant immune responses *(96).* One Of these pathways is the JAK/STAT pathway *(96).* The JAK/signal transducers, and activators of the transcription (STAT)-pathway mediate the signaling of multiple cytokines. The JAK/STAT pathway is one of the mediators of signal transmission from extracellular stimuli such as cytokines, growth factors, and hormones to the nucleus of cells *(96).* Baricitinib is the first generation of JAK1/2 inhibitor targeting the ATPase of JAK, which blocks the intracellular transmission of cytokines through JAK-STATs *(97).*

Baricitinib exerts its anti-inflammatory effects through reversible JAK inhibition., as shown in (Figure 17), Signaling is initiated when cytokines bind to their receptor on the cell membrane *(96).* This results in conformational changes that trigger the activation of associated JAK complexes. JAK activation, in turn, leads to autophosphorylation and subsequent increased JAK kinase activity, and phosphorylation of the intracellular portion of their cognate receptors *(96).* Phosphorylation of the receptor creates a binding site for signaling molecules, especially the STAT family *(96).* Once docked to the

receptor, STAT molecules are also phosphorylated by JAKs. The phosphorylated STATs are then released from the receptor, form homodimers or heterodimers through reciprocal interactions with their newly phosphorylated tyrosine domains and translocate to the cell nucleus where they bind to specific DNA sequences to activate the transcription of the target gene *(94,96).* Cytokine receptors recruit two of the four JAKs to the intracellular domain of the signaling complex (i.e., JAK1/JAK2, JAK1/JAK3, JAK1/TYK2, and JAK2/TYK2 *(97).* (Figure 17). Inhibition of one or both JAK monomers associated with the cytokine receptor is usually sufficient to terminate signal transduction *(98).* JAK1, JAK2, and TYK2 are expressed throughout the human body, whereas JAK3 is primarily expressed by hematopoietic cells in the bone marrow *(98).* The various JAK complexes mediate distinct cytokine signaling pathways.

In general, the Janus kinase and signal transducer and activator of transcription (JAK-STAT) pathway is used by cytokines, including interleukins, interferons (IFNs), and other molecules to transmit signals from the cell membrane to the nucleus. Upon engagement of extracellular ligands, intracellular JAK proteins associated with type I/II cytokine receptors are activated, and phosphorylate STAT proteins, which dimerize, and then translocate to the nucleus to directly regulate gene expression (Figure 18) *(94,99).*

Baricitinib was designed to inhibit JAK1 and JAK2 with less potency for JAK3 selectively *(100).* However, as presented in (Table 6), baricitinib purported selectivity is only evident in cell-free assays but not recapitulated in cell-based assays *(98).*

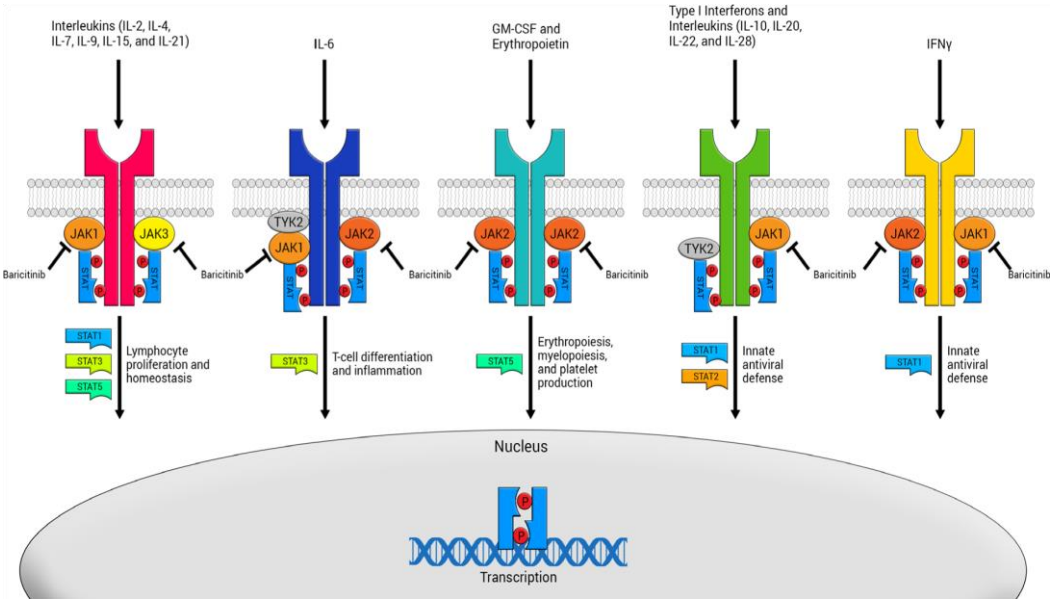

Figure 17. Janus-associated kinase/signal transducers and activators of transcription (JAK/STAT) pathway showing baricitinib inhibition of JAK complexes. GM-CSF = granulocyte-macrophage colony-stimulating factor., IFNc= interferon-gamma (101).

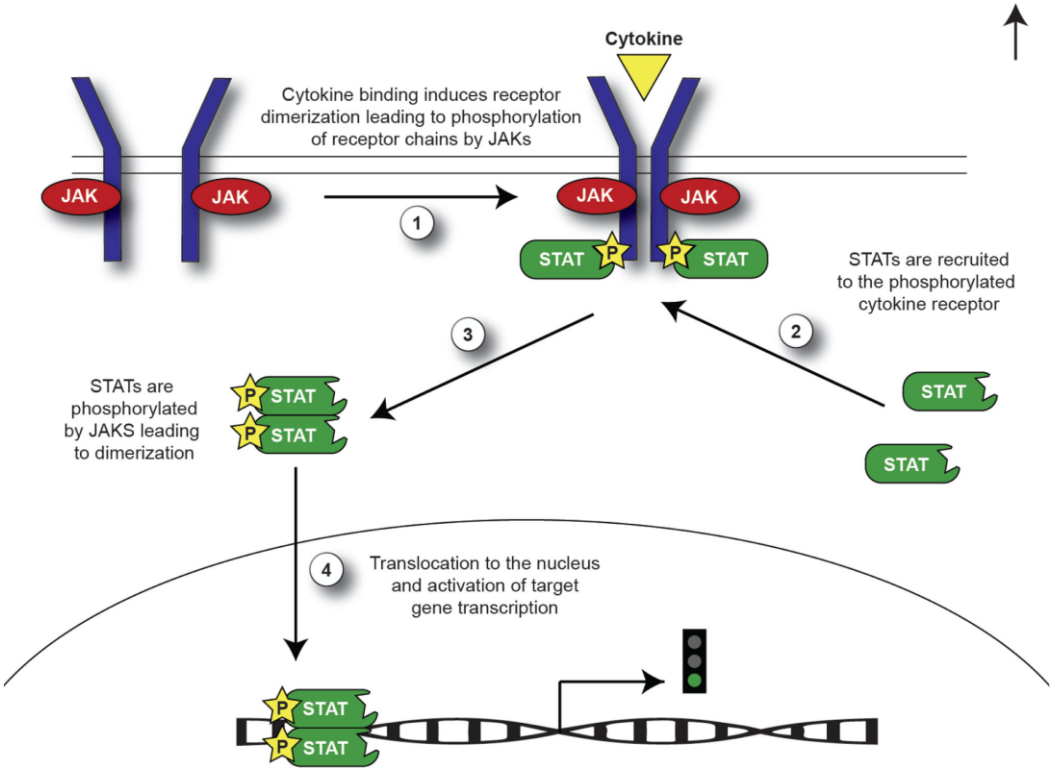

Figure 18. JAK-STAT signaling pathway. JAK inhibitors antagonize JAK protein function and prevent activation of the pathway (95).

Table 6. Anti-inflammatory and Antiviral Activity of Baricitinib (adapted from references (89,98,101)).

JAK enzymes, cell-free	Baricitinib mean IC_{50}, nM	JAK enzyme pair, cell-based[a]	Baricitinib mean IC_{50}, nM
JAK1	5.9	JAK1/JAK2	32.8
JAK2	5.7	JAK1/JAK3	55.4
JAK3	> 400	JAK1/TYK2	71.6
TYK2	53	JAK2/TYK2	69.0

NAK enzymes, cell-free	Baricitinib K_d, nM	NAK enzymes, cell-based[b]	Baricitinib K_d, nM
AAK1	17	AAK1	34
GAK	136	GAK	272

AAK1 = AP2-associated protein kinase 1, GAK = cyclin G-associated kinase, IC_{50} = 50% inhibitory concentrations, JAK = Janus-associated kinase, K_d = dissociation constant, NAK = numb associated kinase, TYK2 = tyrosine kinase 2.

[a]Across multiple cell types, including B cells, CD^{4+} T cells, CD^{8+} T cells, Natural killer cells, and monocytes.

[b] Not directly measured., calculated based on the ratio of cell-based to cell-free inhibition of JAK enzymes.

2.2. Pharmacokinetics

Baricitinib is a medication used primarily for the treatment of rheumatoid arthritis. Understanding its pharmacokinetics is important for optimizing its therapeutic efficacy and minimizing potential side effects. Pharmacokinetics involves studying how the drug is absorbed, distributed, metabolized, and excreted in the body.

This drug is available as 1, 2, and 4 mg film-coated, immediate-release tablets (Figure 19). The oral bioavailability of baricitinib is about 80% with 50% plasma protein binding and 76 L volume of distribution, suggesting uptake

inside tissues *(104).* The peak concentration of the drug is achieved within about 1 h of drug ingestion. CYP3A4 is the main metabolizing enzyme of baricitinib, Studies show that with 75% and 20% renal, and hepatic clearance, are excreted in urine, and feces, respectively, and an elimination half-life of 8 to 12 h *(105).*

Figure 19. Medicinal doses of Baricitinib (102,103).

Plasma protein binding for baricitinib, which is not concentration dependent, is 50%. The mean volume of distribution is 1.1 L/kg, suggesting moderate distribution into tissues (98).

The drug's elimination is mainly through renal pathways, including both filtration and active secretion. Approximately 75% is excreted in the urine (69% unchanged) and 20% in the feces (15% unchanged) (98). Additionally., baricitinib interacts with various drug transporters, which can influence its absorption, distribution, and elimination in the body. Its half-life ranges from 6 to 9 hours in healthy volunteers (98).

Pharmacokinetic (PK) parameters for baricitinib in pregnant or breastfeeding women have not been reported. Currently, it is unknown whether baricitinib crosses the placenta in humans, and there is no data available on its presence in breast milk or its effects on breastfeeding infants. Therefore, the safety and efficacy of baricitinib in these populations remain undetermined. However, skeletal abnormalities and developmental toxicity have been observed in the offspring of pregnant mice exposed to supratherapeutic doses of baricitinib. Effects on fertility in animals have been inconsistent (98).

2.3. Drug-Drug Interactions

Understanding the drug-drug interactions of baricitinib allows for ensuring safety, and effectiveness. It helps prevent serious side effects and ensures the medication works as intended. This knowledge is key to providing personalized and safe medical care, especially for patients with specific health conditions.

Although a small fraction (6%) of baricitinib is metabolized by CYP3A4, coadministration with ketoconazole (a strong CYP3A4 inhibitor) or rifampin (a strong CYP3A4 inducer) has no clinically significant effect on baricitinib PK. As mentioned, baricitinib is a substrate of several drug transporters (P-glycoprotein, BCRP, MATE2-K, and OAT3). Coadministration with cyclosporine (a P-glycoprotein inhibitor) did not result in clinically relevant baricitinib PK changes, However, coadministration with probenecid (a potent OAT3 inhibitor) resulted in a 2-fold increase in renal clearance in AUC (98,100).

Dose reduction in patients using potent OAT3 inhibitors. Based on PK modeling, less potent OAT3 inhibitors, such as ibuprofen, and diclofenac, are expected to have the least effect on baricitinib PK (98,100). Studies investigating the effect of BCRP or MATEK-2 inhibitors have not been reported. Elevated gastric pH and use of proton pump inhibitors do not alter overall baricitinib exposure. Nevertheless, the time to peak plasma concentration is prolonged by up to 2 hours with co-administration of omeprazole. No signal of prolongation of the QT interval (QTc) was observed with baricitinib doses up to 40 mg in healthy volunteers (105-106).

2.4. Safety

When discussing the safety of baricitinib, it's important to consider both its side effects and its impact on laboratory and clinical parameters. Regular monitoring of laboratory parameters is essential during the treatment with baricitinib. Baricitinib drug use causes changes in several laboratory parameters (98,107) (Table 7). Besides these changes have been associated with serious adverse effects, including infections, thrombosis, malignancy, gastrointestinal perforations, and major cardiovascular events (98, 108).

The most common side effects of baricitinib are upper respiratory tract infection (14-22%), headache (11-24%), and nasopharyngitis (11-18%) (98). Many of these have been reported with other JAK-inhibitors and include rapid and sustained decreases in neutrophil, and lymphocyte counts, decreases in hemoglobin, small increases in creatinine (< 0.1 mg/dl),

increases in lipid parameters, elevations in liver enzymes and bilirubin, and increases in creatine phosphokinase (CPK) (109).

Table 7. Laboratory and Clinical Monitoring Parameters While Receiving Baricitinib (adapted from references (98, 100-101, 104).

- Serum creatinine
- Absolute lymphocyte count [a]
- Absolute neutrophil count [b]
- Hemoglobin [c]
- Platelets
- ALT
- AST
- Bilirubin
- CPK
- LDL/HDL (if prolonged use)
- Signs and symptoms of infection
- Signs and symptoms of thromboembolic events

ALT = alanine aminotransferase., AST = aspartate transaminase., CPK = creatine phosphokinase., HDL = high-density lipoprotein., LDL = low-density lipoprotein.

[a] When used for rheumatoid arthritis it is recommended to interrupt therapy when the absolute lymphocyte count falls below 500 cells/mm^3.

[b] When used for rheumatoid arthritis it is recommended to interrupt therapy when the absolute neutrophil count falls below 1000 cells/mm^3.

[c] When used for rheumatoid arthritis it is recommended to interrupt therapy when hemoglobin falls below 8 g/dl.

In clinical trials, a decrease in lymphocyte count has been associated with a higher rate of emergent infections in the treatment of patients with RA (98,107). Lymphopenia is one of the laboratory abnormalities in patients with COVID-19, and lower numbers of lymphocytes are associated with more severe disease (110-111). In addition to being quantitatively reduced, lymphocytes from patients infected with SARS-CoV-2 also show functional exhaustion and decreased functional diversity (112). Patients with preexisting cardiovascular diseases are at increased risk of the most severe COVID-19 complications (113). Furthermore., myocardial injury has been observed in nearly 30% of hospitalized patients with COVID-19 and is significantly associated with higher short-term mortality (113). Although, increases in liver enzymes and bilirubin have been reported with baricitinib, no cases of liver injury satisfying Hy's law have occurred (96,102). Furthermore, higher rates of liver dysfunction have been correlated with more severe COVID-19 (113). Hepatotoxic drug effects may be difficult to detect in these circumstances, and clinicians may need to maintain a high index of suspicion. These data suggest the timing of baricitinib initiation may be important to both avoid amplifying impaired innate immunity and suppress a harmful hyperinflammatory response.

2.5. Clinical Application of Baricitinib in Dermatology

Baricitinib is effective for the treatment of chronic inflammatory diseases, especially rheumatoid arthritis, and systemic lupus erythematosus. Also, it has been reported to positively affect the inflammatory storm associated

with the development of coronavirus disease 2019 *(114).* In addition, a few reports have shown that baricitinib has a beneficial effect in treating interferon (IFN)-related diseases *(115),* diabetic nephropathies *(116)*, and refractory juvenile dermatomyositis *(117).* Baricitinib has shown promising results in dermatology., it has been widely used as a new molecular-targeted therapy. Increasing evidence suggests that baricitinib is effective against Atopic Dermatitis (AD), Vitiligo, Alopecia Areata (AA), and Psoriasis. Many inflammatory dermatoses are driven by inflammatory mediators that rely on JAK/STAT signals, and the use of JAK inhibitors has become a new strategy for the treatment of diseases for which conventional drugs have not been effective (*95).*

2.5.1. Atopic Dermatitis

One of the most common chronic inflammatory skin diseases is AD. Elevated inflammatory cytokines in AD-affected skin include Th2 (interleukin [IL]-4, IL-13, IL-31, IL-5), Th22 (IL-22), Th1, and thymic stromal lymphopoietin (TSLP) *(118).* Cytokines are associated with increased signaling through all four JAKs. IL-4 and IL-13 bind to IL-4 (either the α or γ chain), and IL-13 (α1) receptors to induce JAK1, and JAK3, respectively, activating STAT6. IL-5 binds to the IL-5 receptor (β chain), thus causing the expression of JAK1 and JAK2, resulting in the activation of STAT1, STAT2, and STAT5. In addition, TSLP binds to the α unit of the IL-7 heterodimer, and TSLP receptor, and induces the activation of JAK1, and JAK2, thus activating STAT5 *(119).* Studies have shown that 4 mg of baricitinib combined with glucocorticoids significantly improves the signs,

and symptoms of in adults., moderate to severe AD, with rapid effects, and good safety *(120).*

2.5.2. Vitiligo

The IFN-γ-related chemokine CXCL-10 is involved in the pathogenesis of vitiligo, and IFN-γ signaling is mediated by the JAK/STAT pathway, especially through JAK1, and JAK2. JAK inhibitors can block this pathway, thereby blocking the effects of IFN and CXCL-10. Interestingly, a patient with rheumatoid arthritis and vitiligo showed a reduction around vitiligo lesions after treatment with baricitinib *(121-122).*

2.5.3. Alopecia Areata

AA is a polygenic autoimmune disease characterized by temporary scarless alopecia, and follicular preservation, affecting nearly 2% of the general population at some point in their lives. AA is divided into four subtypes: ophiasis, sisaipho, sudden greying, and diffuse AA. Many inflammatory cells such as $CD8^+$ T cells, mast cells, and natural killer (NK) cells have been observed in AA tissues. These inflammatory cells attack the growing hair and cause hair loss *(123).* The primary treatment for patients with small lesions includes the use of topical glucocorticoids, topical injection of glucocorticoids., contact immunotherapy., and topical use of minoxidil. Systemic use of glucocorticoids and immunosuppressants can be recommended for patients in advanced AA or those displaying large lesions

(124). However, traditional treatments have limited effects in patients with AA totals or universalis. Therefore, molecular-targeted drugs have emerged.

A Phase II randomized controlled study where baricitinib was used to treat adult AA showed that 33.3% and 51.9% of patients with AA had alopecia tool scores of patients with AA had alopecia tool scores of <20 at 36 weeks after oral administration of 2 mg and 4 mg dose, respectively., and baricitinib was well-tolerated *(125).*

2.5.4. Psoriasis

Several studies have explored the use of baricitinib in treating psoriasis. In a randomized, double-blind., placebo-controlled phase 2b study, the safety, and efficacy of baricitinib were evaluated in patients with moderate to severe plaque psoriasis. The study assessed oral doses of 2, 4, 8, and 10 mg, taken once daily for 12 weeks. Results demonstrated that all groups treated with baricitinib showed greater mean changes in their PASI scores from baseline at 12 weeks ($P < 0.05$) compared to the placebo group. Notably, except for the 2 mg dose, the PASI-50 response was higher in the treatment groups than in the placebo group. Additionally., by the end of the study, patients with moderate to severe psoriasis who received baricitinib achieved significant improvements in PASI-75 *(126)*.

In another investigation., the safety and efficacy of baricitinib (BARI) were evaluated in patients with moderate-to-severe psoriasis through a randomized, double-blind, placebo-controlled phase 2b clinical trial. The

study investigated oral doses of 2, 4, 8, and 10 mg. The findings revealed that patients treated with baricitinib for 12 weeks experienced significant improvements in PASI-75, and patient-reported outcome (PRO) measures compared to the placebo group. Over 80% of patients maintained a PASI-75 response through week 24. Additionally, after an additional 12 weeks of treatment, 43% of partial responders, and non-responders achieved PASI-75 at the same or higher dose *(127)*.

In another study., the effects of topical baricitinib., a JAK1/2 inhibitor, were examined using the 12-O-tetradecanoylphorbol-13-acetate (TPA) model of chronic psoriasis in mice. This was the first study to assess the local administration of baricitinib in an in vivo psoriasis model. TPA-induced inflammation was triggered by topical application to both ears. The results demonstrated that topical baricitinib inhibited the expression of TPA-regulated inflammatory markers. Furthermore, baricitinib significantly reduced ear swelling, leukocyte infiltration, epidermal cell proliferation, and angiogenesis in the dermis *(128)*.

There is also a case report of a patient with rheumatoid arthritis who developed inverse psoriasis during treatment with Baricitinib, which has been suggested to be due to increased expression of IL6, IL8 (C-X-C motif ligand [CXCL]-8), and IL36 gamma gene induced by baricitinib *(129)*.

Like paradoxical psoriasis caused by TNF-alpha inhibitors, in another report, baricitinib was shown to induce a new onset when administered to a patient with RA who also developed psoriasis. This mechanism is probably due to disturbing the balance of cytokines to increase the expression of IL-6, IL-8, and IL-36 gamma genes *(129)*.

2.6. Perspectives of the clinical research on topical JAK inhibitors

Baricitinib is a first-generation JAK1/2 inhibitor. JAK is at the end of the cytokine receptors located in the cell membrane and controls the signal transduction of many cytokines, such as the IL-6, IL-10, IL-3, and IL-5 families *(94).* Each cytokine receptor is linked to two parallel isomers of JAK that exist as homodimers or heterodimers. When cytokines bind to their receptors, JAK phosphorylation occurs, which leads to the phosphorylation of STAT proteins in cells. Subsequently, these proteins are transported to the nucleus to act directly on the cell's DNA and ultimately regulate gene expression *(94).* Signal transmission is directed from the outside to the inside of the cell. Chronic inflammatory diseases associated with upstream cytokine disorders can be treated with baricitinib.

Clinical disease screening of patients before the application of JAK inhibitors, and continuous monitoring during application is essential *(130).* Some experts believe that more targeted second-generation JAK inhibitors for only one subtype or topical application show better effects and lower adverse reactions *(131-132).*

New molecularly targeted therapeutics are changing dermatologic therapy. Numerous inflammatory dermatoses are driven by soluble inflammatory mediators that rely on JAK-STAT signaling, and inhibition of this pathway using JAK inhibitors may be a useful therapeutic strategy for these diseases *(95).*

The use of topical JAK inhibitors in AD and vitiligo has been investigated. One of the most common drugs to treat both diseases is the drug Opzelura, which

contains the active ingredient ruxolitinib. This drug belongs to the group of drugs known as Janus kinase inhibitors. The active substance in Opzelura blocks enzymes known as Janus kinase (JAK) 1 and 2, which are involved in the activity of a substance called interferon-gamma (IFN-gamma) it works.

It is not recommended to use this drug together with biological therapy drugs, other JAK inhibitors, or strong immunosuppressants such as azathioprine or cyclosporine, and it cannot be used on the lips, eyes, mouth, or vagina *(133-136).* When possible, immunizations should be performed before initiating therapy with JAK inhibitors *(99,137).*

3. The skin

3.1. Structure and Function of the Skin

The skin is the largest organ of the human body *(138).* The skin is a complex organ that is the body's first protective barrier. The clarification of skin structure, mainly concerning its barrier function., has been studied by many researchers since the 1960s *(139-143).* It is a multi-layered tissue, and its main function is to protect the body from external conditions by acting as an effective barrier against the absorption of exogenous particles *(144-146).*

The skin is a protective organ that performs a wide variety of functions, such as protection against external aggression, thermoregulation., waterproofing, absorption of ultraviolet (UV) radiation, secretion, vitamin D production, safety, and immunological defense against pathogenic microorganisms, as well as the detection of sensory stimuli *(147-149).* It constitutes an organ

that functions permanently and is one of the structures with the largest surface area (1.5 to 2 m^2), and body weight (3.5 to 4 kg), representing 7% of the total weight of the human body. It forms an effective barrier against unpleasant stimuli from the outside, and at the same time, functions as a semi-permeable membrane that helps the body maintain its proper hydration.

To adapt to environmental changes, the skin has evolved into a dynamic system with homeostatic capabilities, allowing it to cope with varying conditions such as age, race, climate, sex, and health status *(150).*

Male skin is generally thicker than that of women and children, increasing in thickness until around the age of 30 or 40, after which it begins to progressively thin *(149).* Skin is an important target as well as a major obstacle for dermal drug delivery *(151).*

Anatomically the skin is composed of three well-differentiated layers: the epidermis, dermis, and hypodermis, each performing distinct but interrelated functions *(148)* (Figure 20).

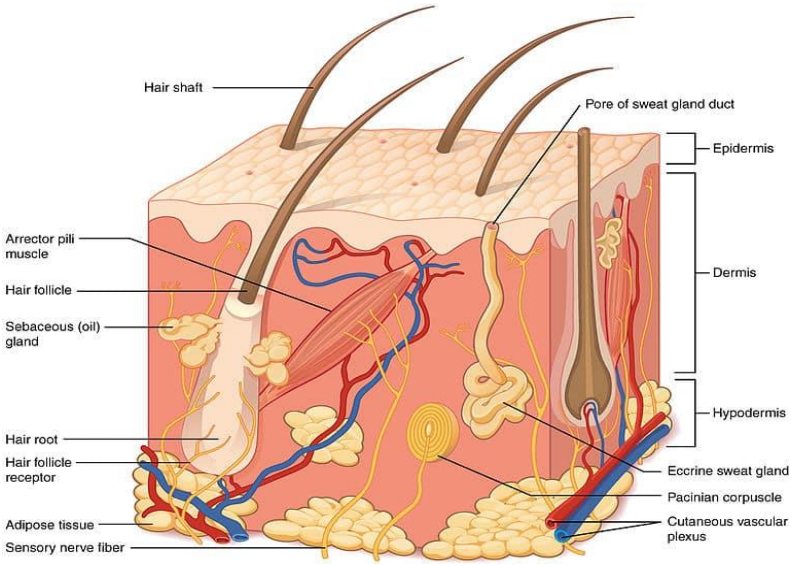

Figure 20. The skin is comprised of three main layers: epidermis., dermis., and hypodermis (152).

3.1.1. Epidermis

The epidermis is the outermost layer of the skin, measuring 120-200 microns in thickness. It consists of four types of cells: keratinocytes (comprising 95% of the epidermis), melanocytes (which transfer pigment to keratinocytes), Merkel cells (neurosecretory cells with sensory functions), and Langerhans cells (immunocompetent dendritic cells with an immunological function) (148). The epidermis layers describe the different stages of cellular life in the is divided into four distinct layers: the stratum germinativum (basal layer), stratum spinosum (spinous or spiny layer), stratum granulosum (granular layer), and the Stratum lucidum (stratum corneum) (152) (Figures 21,22) shows the different layers of the epidermis.

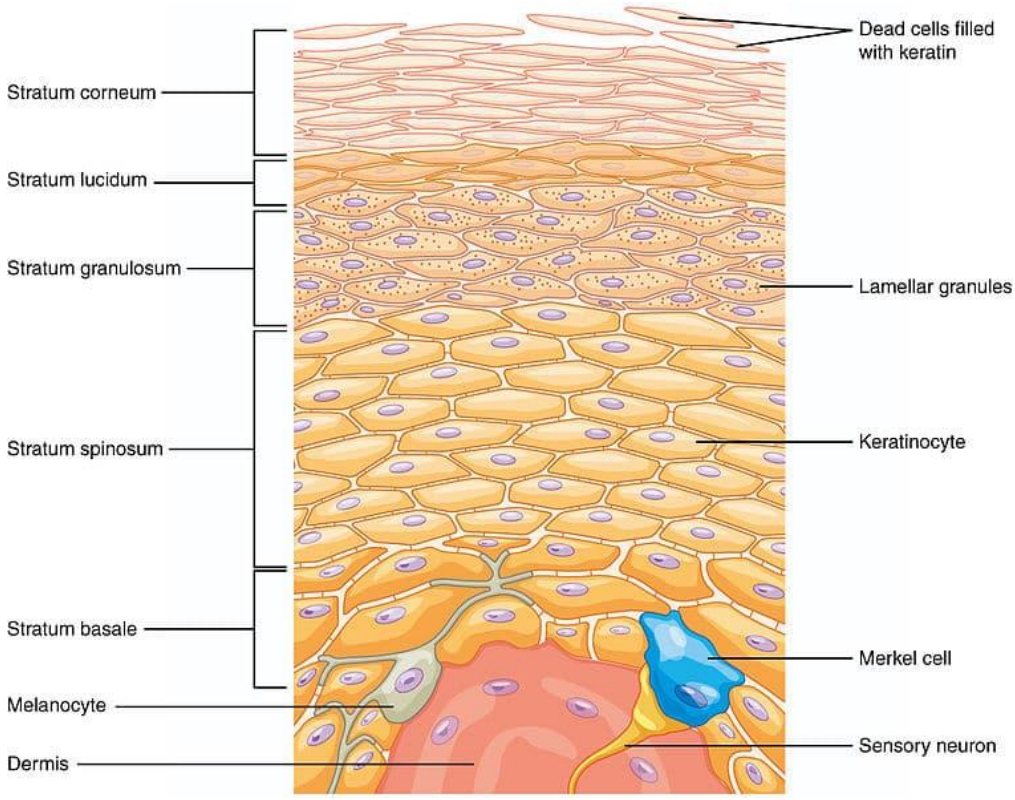

Figure 21. The epidermis layers are formed by keratinocytes at different maturation levels. (152).

The basal layer, located deepest within the epidermis, is composed of a single row of cells connected by desmosomes, on which keratin is inserted. It is supported at the base by hemidesmosomes, resting on the dermal basal membrane. The basal cell layer constitutes the germinal layer of the epidermis under normal conditions, and after each division, so that half of the resultant cell population contributes to epidermal development. Under normal conditions, differentiated keratinocytes take about 2 weeks to reach the stratum corneum, and another 2 weeks to shed *(153).* The maturation or differentiation process of basal keratinocytes involves transforming these basal cells into fully keratinized cells, known as corneocytes, which make up the stratum corneum *(81,153).*

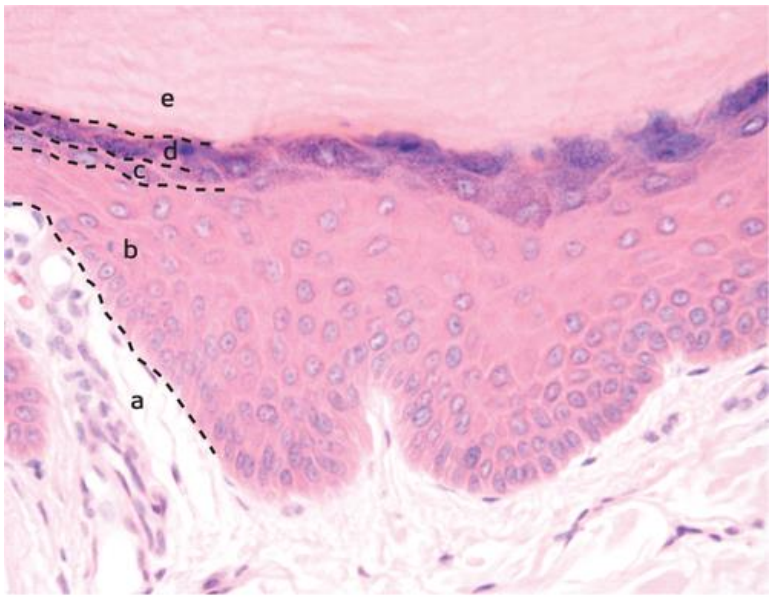

Figure 22. Layers of the epidermis (h-E40x). This image corresponds to a thick section of the skin., showing the different layers of the epidermis: (a) basal., (b) spinous., (c) granulosa., (d) lucid., and (e) cornea (155).

The spinous layer consists of 3 to 10 rows of polygonal cells that gradually flatten as they approach the surface (81).

The granular layer comprises between 1 and 4 rows of flattened cells containing granules. Along with the stratum lucid, they form the pre-corneal stratum which is metabolically inactive (148,153).

And the outermost layer is the stratum corneum formed between 15 and 25 flattened cells without a nucleus cell characterized by the thickening of the cytoplasmic membrane (153).

The process of keratinization or differentiation of epidermal keratinocytes consists of the transformations of these cells from active mitosis from the metabolic point of view to a flattened form without a nucleus that peels off the surface of the skin, in a period of 'about thirty days. Throughout this

process and depending on the state of differentiation achieved (Figure 23), the cell synthesizes various types of keratins, which are the main structural proteins of keratinocytes and allow the appropriate modulations of the cytoskeleton., it also synthesizes several lipids that allow the permeability of the epidermis to be controlled. This important protein synthesis., the formation of numerous intercellular junctions, and the constant recycling of cells make it possible to maintain a sufficiently elastic, cohesive, and waterproof epidermis. However, this balance is fragile, and a defect in keratinization due to a genetic mutation, an immune disturbance, or environmental aggression can have serious consequences on the homeostasis of the epidermis and the barrier function of the stratum corneum *(150,153).*

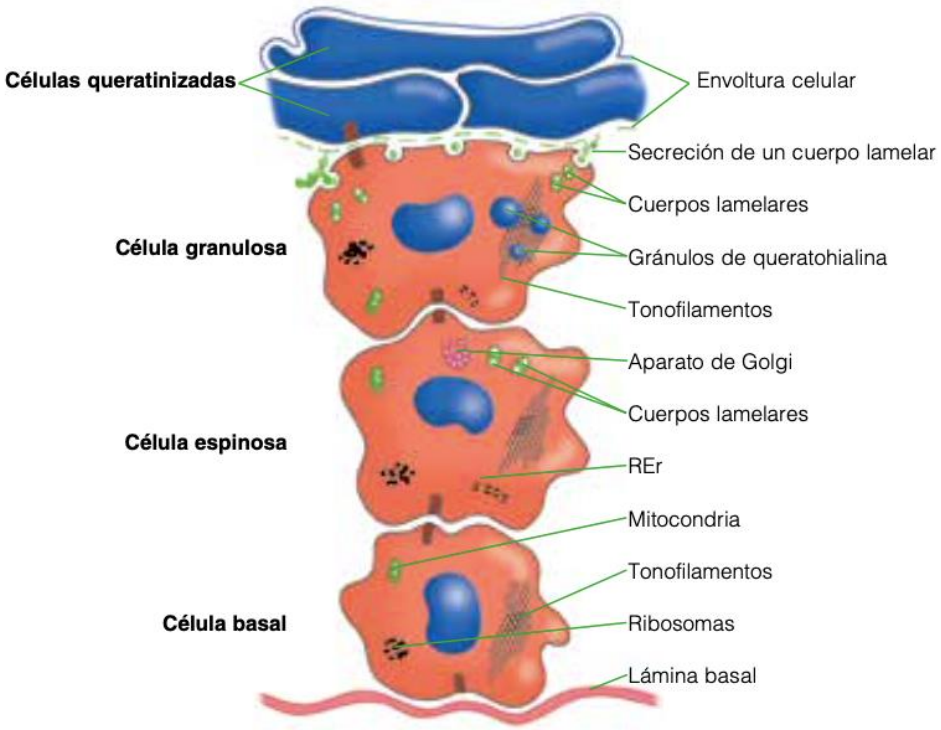

Figure 23. Through successive multiplication and differentiation., the keratinocytes ascend from the basal layer to the skin surface., building during transit the other layers of the epidermis (spinous., granulosa., and cornea) (148).

The epidermis is a living tissue, except for the stratum corneum, which is the outermost layer. The epidermis is not vascularized, and nutrients diffuse through the dermo-epidermal junction to maintain its viability *(155).*

The skin barrier protects the body by providing mechanical protection against drying, and the penetration of dangerous substances, and microorganisms. The stratum corneum acts as an important part of the barrier function for local drug penetration. Various models have been proposed for SC simulation. The simplest model is defined as a "brick and mortar" structure. The stratum corneum consists of corneal cells (“bricks”) and an intercellular lipid matrix (“mortar”) that is primarily responsible for barrier function *(156).* SC cells are called corneocytes. There are 15 to 20 layers of the cornea with a thickness of 10 to 15 µm, but when hydrated., the stratum corneum grows significantly, and its thickness may reach up to 40 µm, indicating long-term permeability. These cells are dense., functionally dead., and nucleated. Lipids form two layers around the cornea of cells. Intercellular lipids are a mixture of fatty acids, ceramides, cholesterol, cholesterol esters, and a small fraction of cholesterol sulfate *(157-159).* The stratum corneum is the main layer that limits drug penetration through the skin due to its barrier properties and water resistance *(155).*

Three different pathways allow substances to cross the skin barrier *(160).* For the passive diffusion of a molecule., there are three main permeation pathways across the SC. The first and most important known pathway is the intercellular penetration pathway. The second path is the path of follicular penetration. The intercellular permeation pathway., where substances pass through corneal cells, and lipid bilayers, currently appears to be unimportant *(161-162).*

The difference between penetration and skin permeation is important. Buist and colleagues have defined them as follows *(163).* The movement of a chemical substance from the skin's outer surface into the epidermis, but not necessarily into the circulatory system, is called Dermal penetration. Penetration from one layer to another, functionally and structurally different from the first layer, is called Skin permeation.

3.1.2. Dermis

This layer constitutes the support of the epidermis and comprises a fibrous component (collagen and elastic fibers) plus the fundamental substance. Collagen represents 80-85% of the dry weight of the dermis and is the main determinant of its tensile strength. Elastic fibers represent 2-4% of the extracellular matrix and consist of elastin, and microfibers, providing skin elasticity. It also contains various glycoproteins, such as fibronectins, fibulins, and integrins, which facilitate cell adhesion and motility. The fundamental substance is composed of several macromolecules of glycosaminoglycans/proteoglycans, which have the fundamental role of maintaining the hydration of the dermis, especially due to the union of water (which constitutes 60% of the total weight of the dermis) with the 'hyaluronic acid. The cellular component of the dermis is made up of fibroblasts, and various cells involved in immune, and inflammatory processes, as well as epidermal appendages, and vascular structures *(150).*

3.1.3. Hypodermis

It is the deepest layer of the skin, containing fat cells or adipose tissue that insulate the body and help it conserve heat. This layer is composed of loose connective tissue and many fibers are attached to those of the dermis forming anchor points, thus fixing the skin to the underlying structures (fascia, periosteum, or perichondrium). Poorly developed attachment points cause the skin to shift and form folds, whereas highly developed or numerous attachment points, as observed on the soles of the feet or scalp, render the skin nearly immovable. The thickness of the hypodermis is very variable, depending on location, body weight, sex, or age. This layer also contains hair follicles, sensory nerves, and blood vessels *(150).*

3.2. Percutaneous Absorption

The number of skin formulations has increased in recent decades. The main reason for their success is that they have countless advantages. For example, these include non-invasive treatment, gastrointestinal tract protection, and hepatic first-pass metabolism avoidance. Topical semi-solid preparations are complex formulations. The physical properties of the product depend on several factors, including the particle size of the dispersed particles, surface tension between phases, fractional distribution of the drug between phases, and rheological behavior. These characteristics together determine the laboratory emission profile, along with additional characteristics. The amount

of active pharmaceutical ingredient (API) released in vitro is an essential characteristic of a product *(156).*

There are several types of methods for penetrating drug delivery systems. One of the most important methods is topical and transdermal formulation, usually for drug delivery to the skin, and systemic application. Modeling penetration into the skin layer and penetration through the skin is a complex challenge. A device with formulation characteristics affects how the most effective system is examined *(156).*

Various products, including creams, ointments, and gels, are regularly used by patients. Topical formulations deliver the active ingredient to different layers of the skin. Therefore, various diseases can be prevented and/or treated.

The selection of APIs for transdermal delivery should be based on many factors, including physicochemical properties, drug interactions with membranes, and pharmacokinetic aspects. The ideal physicochemical characteristics of a drug selected for dermal administration are low molecular weight (<600 Da). Low melting point (<200℃), which corresponds to good solubility. A high but stable partition coefficient because very high partition coefficients may increase drug retention, thereby preventing drug clearance from the skin, and of course, solubility in water, and oil to achieve an appropriate concentration gradient, and increase spreading power on the skin *(157-160).*

Permeation through the skin is also affected by biological factors. Despite factors such as skin hydration level, age, gender, skin surface location, abnormalities caused by disease or injury, and previous treatment, there is

substantial variation in the skin barrier *(161-165).* The skin's hydration level must be balanced, and the required volume of water must be sufficient for the proper functioning of the skin. If hydration is increased, permeability may improve *(166).* Age affects skin penetration. Baby skin and damaged skin have higher permeability.

The permeability of the drug is regulated through the stratum corneum, and the carrier or vehicle used. A vehicle can improve the physical condition and permeability of the skin by the effect of hydration or changing the structure of the lipid bilayer.

3.2.1. Stages of Percutaneous Absorption

The onset, duration, and depth of the therapeutic effect depend on the effectiveness of three sequential processes:

I. The release of the active pharmaceutical ingredient (API) from the carrier system is influenced by Interactions between the drug, skin, and vehicle.
II. The penetration/permeation of the API into the stratum corneum (SC) or deeper layers of the skin.
III. And the effect at the target point.

The release of an API from drugs applied to the skin and its transport into the systemic circulation is a multistep process that includes the release of the API from the preparation., followed by the partitioning of the drug into the

stratum corneum, and its diffusion within the stratum corneum. Subsequently., there is a distribution of the drug from the stratum corneum to the layers of the living epidermis., which is followed by the drug's diffusion through the layers of the living epidermis into the dermis. Finally, the drug may be absorbed by the vessels, resulting in entry into the systemic circulation (Figure 24) *(167).* All these processes collectively determine the performance profile of a product *(156).*

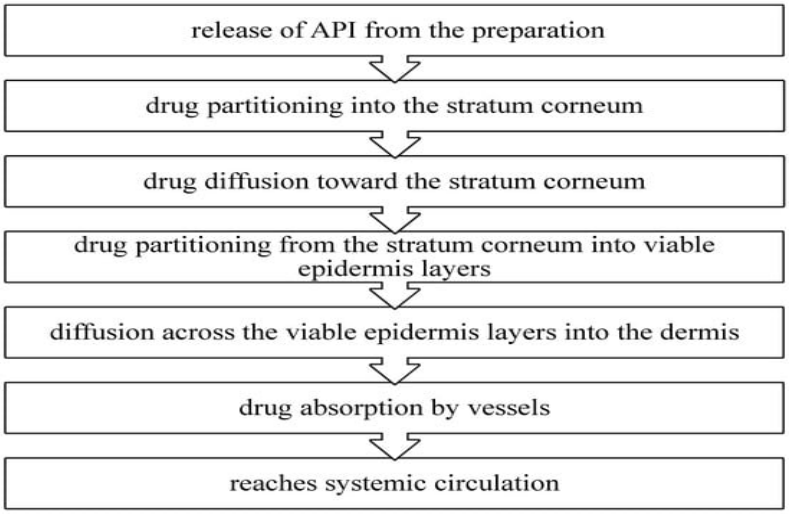

Figure 24. Drug transport across the skin (167).

4. Insights for Evaluating Topical Formulations

The evaluation of topical formulations is a multifaceted process that encompasses various aspects of drug development and testing. This section provides a comprehensive overview of the critical factors and methodologies involved in assessing the efficacy, safety, and quality of topical drug products. By understanding these key elements, researchers and developers can ensure

that their formulations meet the necessary standards for clinical, and commercial success.

4.1. Drug Bioavailability

Drug bioavailability is a crucial parameter in the evaluation of topical formulations. It refers to the extent and rate at which the active pharmaceutical ingredient (API) is absorbed through the skin and becomes available at the site of action. However, several factors influence bioavailability. For instance, many drug molecules show poor permeability because of their unfavorable physicochemical, and chemical features, which are difficult to change. To address this challenge., an external excipient may be added to increase drug solubility or enhance the permeation transiently *(168).*

4.1.1. Transcutol®

One such excipient that has gained significant attention for its ability to enhance drug bioavailability is Transcutol®. Transcutol® (diethylene glycol monoethyl ether, DEGEE), CAS # 111-90-0, is a clear, colorless, moist liquid with a mild pleasant odor (Figure 25). It is produced from the condensation of ethylene oxide and alcohol followed by pure distillation *(169).* DEGEE is soluble in water and miscible in acetone, benzene, chloroform, ethanol (95%), ether, and pyridine. It is partially soluble in vegetable oils and insoluble

in mineral oils *(170).* Due to its properties as a strong solvent with low toxicity., DEGEE has a long history of safe use as a solvent in many products including pharmaceutical, cosmetic, and food applications. Accordingly, it is used as a vehicle in the formulation or manufacturing process of medicines, cosmetics, and food additives *(171).* Transcutol® is also a highly effective penetration enhancer used in topical formulations. It works by temporarily modifying the skin barrier., thereby increasing the permeability of the API.

Figure 25. Chemical structure of diethylene glycol monoethyl ether (C6H14O3., CAS No. 111-90-0) (171).

4.2. Guidelines of Drug Release Testing

Drug release testing is a critical component in the development and quality control of pharmaceutical products. It ensures that the drug is released from its dosage form consistently and predictably., which is essential for its efficacy and safety. Several guidelines provide recommendations for conducting in vitro release testing (IVRT) studies, which are pivotal in assessing the performance of topical drug products. Key Guidelines in this field include the European Medicines Agency (EMA), Guidelines which provides comprehensive guidelines on the quality and equivalence of topical products. Annex 1 of the draft guideline specifically addresses the requirements for IVRT studies. These guidelines emphasize the need for robust and scientifically sound methods to ensure the reliability and reproducibility of the results. EMA Guideline *(172),* and the FDA's Guidance document on in

vitro release test studies for topical drug products outline the procedures and criteria for conducting IVRT studies. This document highlights the importance of method validation and the need for rigorous testing to ensure that the drug release profiles are consistent with the intended therapeutic outcomes FDA Guideline *(173)*.

Several points should be mentioned about IVRT:

- Scope of IVRT

This appendix provides information for in vitro release (IVRT) of semisolid drug products (such as creams, gels, or ointments) and liquid suspensions. Some topical products are off-limits for IVRT, but other lab tests may be applicable: simple liquid solutions., topical powders., and other nonstandard topical formulations (eg., foams).

- IVRT logic

A quasi-infinite dose IVRT using diffusion cells evaluates the rate and extent of diffusion of an active ingredient in a proposed formulation, where the following parameters must be determined:

This Guideline suggests calculating the drug release rate (R) as the slope of the cumulative amount of active substance released versus the square root of time for the linear portion of the drug release profile., this kinetic profile is known as Higuchi's model., which is common in semisolid products., yet., other kinetic models describe the drug release process such as Zero-Order, First-Order, Korsmeyer-Peppas Model, Weibull Model *(174 -175)*.

The cumulative amount (A) of active substance released, usually expressed in units of mass per surface, at the last sampling time of the linear section. Lag time (if applicable).

- Study design

An IVRT pilot study to compare test and comparator products to confirm the suitability of the selected membrane, and to confirm the test conditions should be justified concerning:

A. Membrane selection:

In this case., it must be ensured that the product and the receiving medium remain separate to ensure that the tested formulation remains unchanged during the test period. The membrane should not limit the diffusion rate of the active ingredient. Also, the membrane must be compatible with the formulation of the medicinal product, and not be attached to the active ingredient.

B. Choosing the receiving environment:

The sink conditions must be confirmed. Acceptable sink conditions are conditions where the maximum concentration of the active substance in the receiving medium obtained during the test does not exceed 30% of its maximum solubility in the receiving medium. Sink conditions usually occur at a volume of medium that is at least 3-10 times the saturation volume and backscattering of the receiving medium must be minimized to avoid deformation. The applied drug product pH of the receptor medium should be kept constant during the release test.

C. Sampling time

(At least hourly) and experimental conditions (such as device., temperature., and mixing speed) should be defined. The duration of IVRT should be sufficient to determine the release profile., ideally at least 70% of the applied active ingredient is released. At least 6 time points should be obtained in the linear portion of the drug release profile, including the first sample immediately after steady-state drug release. It is unconventional to choose IVRT sampling times of less than 4 hours of study duration. This may occur in situations where the steady-state dose of the product is greatly reduced by 4 h, after which the release kinetics are no longer linear (when plotted against the square root of time). Quasi-infinite dose formulation evaporation effects must be minimized.

D. The temperature

The temperature is in the range of 32°C ± 1°C across 6 hours, the results can support a demonstration that the equipment is qualified to perform its function in an IVRT method for which a method parameter is the control of membrane surface temperature in the range of 32°C ± 1°C across 6 hours. While an IVRT membrane surface temperature in the range of 32°C ± 1°C is appropriate for topical products applied on the skin., for topical products applied on mucosal membranes (e.g., a vaginal gel) the relevant IVRT membrane surface temperature would be 37°C ± 1°C.

In such instances, it would be appropriate to explain the efforts that were made to optimize the IVRT method. Information on the empirical solubility and stability of the drug in the receptor solution, as well as information on the linearity and precision of the resulting drug release rate in an IVRT should

be provided to help explain the selection of a receptor solution for the test method *(172 -173).*

4.3. General Guidelines of Skin Penetration Testing

Today, the regulation of skin and transdermal drugs has received much attention. Many documents are available for dermal absorption studies (Figure 26). These documents promote a coordinated way of conducting dermal and transdermal studies. The Organization for Economic Co-operation and Development (OECD) published several issues on this topic, including Guidance Notes on Dermal Absorption (No. 156) *(171),* Test Guidelines 427, which covers in vivo methods, provides a comprehensive framework for conducting skin absorption studies in live animals *(176),* and 428 which outlines the in vitro method for skin absorption testing. This guideline provides detailed procedures for using human or animal skin samples in diffusion cells to measure the absorption of test substances *(177),* and a guidance document for skin absorption studies *(178).* Some other documents include the World Health Organization International Program on Chemical Safety (WHO/IPCS) Environmental Health Criteria 235 *(179).* European Center for Toxicology and Toxicology of Chemicals (ECETOC) Monograph 20 *(180).* The US Environmental Protection Agency (USEPA) reports on dermal exposure assessment *(181),* and the European Food Safety Agency (EFSA) guidance on dermal absorption for plant protection products *(182-183).* These documents provide rules, and explanations on how to perform skin absorption assays., there are two recommended methods for skin

absorption. It is one of the diffusion cells, and tape separation methods. The guidelines also highlight the need for appropriate controls in using human skin samples and stress the importance of validating the analytical method used in these studies to ensure reliable, reproducible results.

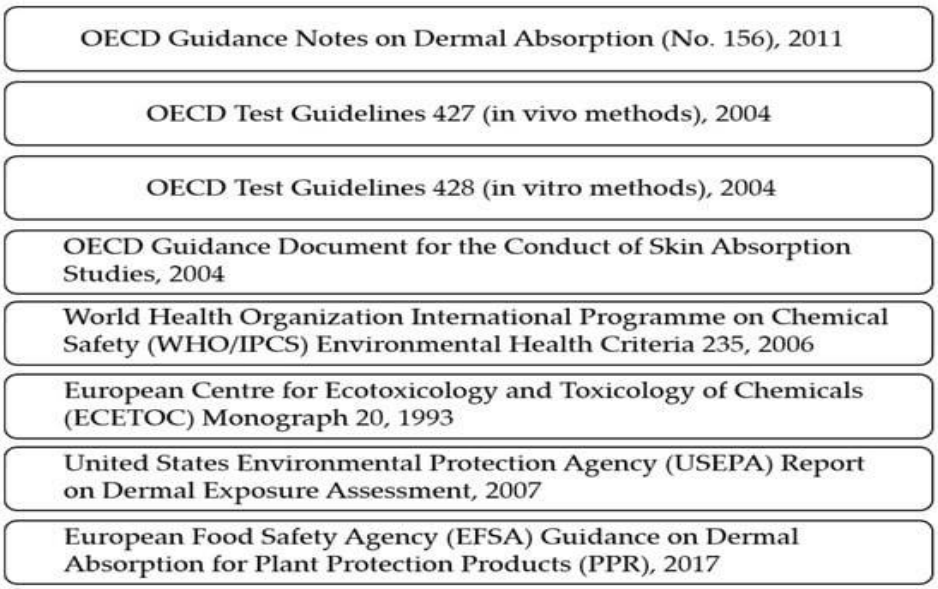

Figure 26. Guidelines for modeling` dermal penetration/permeation (167).

4.4. Validation of Analytical Methods

As mentioned, ensuring that the analytical methods used in IVRT and IVPT studies are validated is crucial because this guarantees that the results are accurate, reliable, and consistent. Using validated methods helps minimize errors, fulfill regulatory requirements, and confirm that the methods are suitable for their intended purposes. This process ultimately enhances the credibility and integrity of the research findings. Validation is based on International Council for Harmonization (ICH) Q2 (R1) guidelines on parameters such as linearity, specificity, system suitability, precision,

accuracy, quantification, robustness, limit of detection (LOD), as well as the limit of quantitation (LOQ) (86,184).

4.4.1. Linearity and Range

The analytical method's linearity is its ability to extract test results that are directly proportional to the analyte concentration in the samples within a certain range (185).

It is a general analytical method for a set of studied drug solutions with different concentrations (usually a range of time intervals with high and low concentrations) under experimental conditions. Calibration curves for each drug solution are constructed by plotting the fluorescence quenching values against the respective drug concentrations. Linear regression analysis of the obtained results and different analytical parameters are calculated.

To determine the drug content in different commercial forms, a general analytical method is used, which has a suitable level of accuracy, precision, and linearity, and the results obtained are statistically compared with the reported methods (186-190).

4.4.2. Accuracy and Precision

The accuracy (standard summation method) of an analytical method refers to the technique's ability to measure the value of a quantity relative to its

"true" value. Which accepted a real or accepted reference value and an estimated value (86). Usually, the accuracy of each concentration level is expressed as the average percent deviation or relative error (RE%), and to check the accuracy of the method, recovery studies are also performed by adding a standard drug solution to the pre-analyzed sample solution (191).

The precision expresses the repeatability of the responses of repeated measurements. The more the number of answers and the less the error, the better the accuracy (86).

4.4.3. Limit of Detection and Limit of Quantification

Limit of detection (LOD) is the lowest amount of analyte in the sample that can be detected. The limit of quantification (LOQ) is the lowest amount of analyte in the sample that can be quantitatively determined (192).

Different analytical methods have been developed to evaluate the amounts of drugs such as, ultraviolet-visible (UV) - spectrophotometry and high-performance liquid chromatography (HPLC). Despite the advantages like reliability., repeatability., and high sensitivity, the HPLC method has disadvantages like high cost and time consumption (193). UV-spectrophotometry is simple, rapid, and easy to use with disadvantages like low sensitivity and unreliable (192). Fluorimetry is a simple, inexpensive, fast, and reproducible method used to estimate many compounds (195-196).

Being economical and precise, the developed method may conveniently be adopted as an alternative method for the routine analysis of baricitinib in bulk and pharmaceutical dosage forms (197).

The proposed methods can be readily used without the need for expensive apparatus, and complicated steps.

Similar spectrofluorimetric methods have been widely applied in pharmaceutical analysis due to their high sensitivity, selectivity, and stability (198).

4.5. Animal Models for Evaluating Topical Formulations in Psoriasis Research

Animal experimental research is important in dermatology, particularly in the development of new therapeutic approaches for chronic diseases such as psoriasis. Understanding and treating this condition requires comprehensive research, which often includes animal models. Animal models help researchers unravel the complex mechanisms underlying dermatological diseases. By studying these models, scientists can observe the progression of skin conditions in a controlled environment, providing insights into the biological processes involved (199-202).

Before new treatments can be tested in humans, they must be evaluated for efficacy and safety. Animal models offer a preliminary platform to test the therapeutic potential of new formulations. This step is essential to ensure that treatments are both effective and safe for human use. Certain animal models, such as the Imiquimod (IMQ) mouse model for psoriasis, closely mimic human skin conditions (203). These models allow researchers to study

the effects of treatments in a way that is highly relevant to human patients, increasing the likelihood of successful translation to clinical practice (200).

The use of histological studies in animal models has led to significant advancements. By examining tissue samples from these models, researchers can observe cellular and molecular changes that occur in response to various conditions or treatments (204). This helps in understanding disease mechanisms., identifying potential therapeutic targets, and evaluating the efficacy and safety of new treatments.

Despite the indispensable nature of animal research, it is governed by strict ethical guidelines designed to ensure the humane treatment of animals. These guidelines mandate that researchers prioritize the welfare of the animals, minimizing pain and distress as much as possible.

4.6. Tolerance Studies from Lab to Clinic

Tolerance studies of topical formulations are essential in both preclinical and clinical research. These studies are conducted to evaluate the safety and tolerability of new topical treatments when applied to the skin. In animal models., researchers apply the formulations to the skin of experimental animals to observe any adverse reactions, such as irritation, inflammation, or allergic responses. These studies help identify potential risks and ensure that the formulations are safe for further testing in humans (205).

In human trials, tolerance studies involve applying topical formulations to the skin of volunteers under controlled conditions. Researchers monitor the volunteers for any signs of adverse reactions and assess the overall

tolerability of the treatment. These studies are crucial because they provide valuable information about the safety profile of the formulations, helping to ensure that they do not cause harm when used by patients. By identifying and addressing any potential issues early in the development process, tolerance studies contribute to the development of safe and effective topical treatments for various skin conditions.

OBJECTIVES

Objectives

The main objective of this Thesis is to develop a formulation containing baricitinib to manage psoriasis. To achieve this goal, we set the work into two specific objectives, each with sub-objectives:

1. Firstly, to study the solubility of baricitinib in different excipients and media as a preliminary and pre-formulation study.
    a. Based on the baricitinib's solubility select the most suitable excipients for elaborating a lipid-based formulation.
    b. To investigate the uptake through different tissues, and to determine the efficiency of the drug extraction method from the tissues (recovery).
    c. To develop and validate an adequate analytical method for determining baricitinib in samples obtained within the frame of this Thesis.

2. Upon gaining knowledge of the solubility and permeability profile of baricitinib, and having determined the most suitable excipients., this Thesis aimed to develop the lipid-based formulation and its subsequent characterization and evaluation. To do so, we set the following sub-objectives:
    a. To design and optimize the lipid-based formulation by investigating different proportions of the selected excipients.
    b. To characterize the selected formulation in terms of physical, chemical, rheological, and biopharmaceutical features.

c. To examine the formulation's efficacy using an imiquimod-induced psoriasis mice model.
d. To investigate the differences regarding the permeability of baricitinib between healthy and psoriasis skin.
e. To explore the tolerability of the developed formulation by in vivo methods.

RESULTS

Results

The results of this Thesis are reported in two scientific journal articles according to the two specific objectives set. And, both articles have been published in the first quartile (Q1) indexed international journals:

Article 1

Mohammadi-Meyabadi, R., Beirampour, N., Garrós, N., Alvarado, H.L., Limón, D., Silva-Abreu, M., Calpena, A.C., Mallandrich, M. Assessing the Solubility of Baricitinib and Drug Uptake in Different Tissues Using Absorption and Fluorescence Spectroscopies. Pharmaceutics 2022., *14*(12)., 2714. https://doi.org/10.3390/pharmaceutics14122714

Article 2

Mohammadi-Meyabadi, R., Mallandrich, M., Beirampour, N., Garrós, N., Espinoza, L.C., Sosa, L., Suñer-Carbó, J., Rodríguez-Lagunas, M.J., Garduño-Ramírez, M.L., Calpena, A.C. Lipid-based formulation of baricitinib for the topical treatment of psoriasis. Pharmaceutics 2024., 16(10)., 1287. https://doi.org/10.3390//pharmaceutics16101287

These results are presented next accompanied by a brief summary.

Article 1

pharmaceutics

MDPI

Article

Assessing the Solubility of Baricitinib and Drug Uptake in Different Tissues Using Absorption and Fluorescence Spectroscopies

Roya Mohammadi-Meyabadi [1,2], Negar Beirampour [1], Núria Garrós [1], Helen Lissette Alvarado [1,2], David Limón [2,3], Marcelle Silva-Abreu [1,*], Ana Cristina Calpena [1,2] and Mireia Mallandrich [1,2,*]

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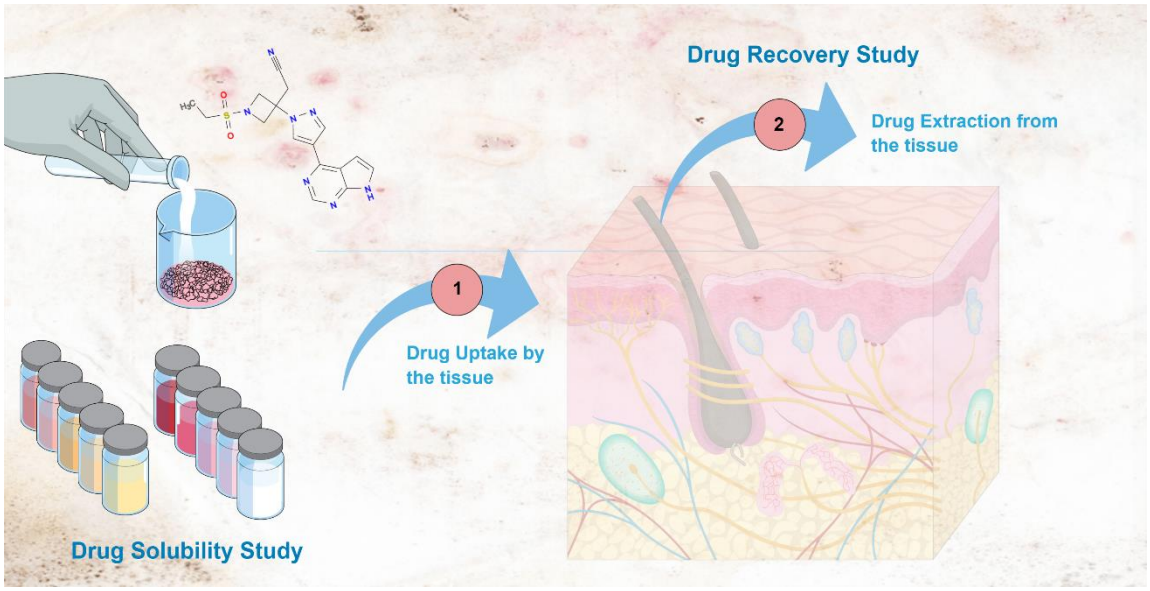

Summary

The limited water solubility of baricitinib (BCT) poses challenges for developing new topical drug formulations. This study aimed to evaluate BCT's solubility in various solvents, including Transcutol, a biocompatible permeation enhancer that is water-miscible. Additionally, the study assessed BCT uptake in human skin, and porcine tissues (sclera, cornea, oral, sublingual, and vaginal) and subsequently extracted the drug from these tissues to determine recovery using in vitro techniques. Analytical methods were developed and validated for quantifying BCT in Transcutol using absorption and fluorescence spectroscopies, which complement each other and allow for drug detection across a wide concentration range. The results indicate that Transcutol enhances BCT solubility and enables its penetration into the studied tissues. BCT solutions in Transcutol remained stable for at least one week, suggesting that Transcutol could be a suitable solvent for further development of topical formulations.

pharmaceutics

Article

Assessing the Solubility of Baricitinib and Drug Uptake in Different Tissues Using Absorption and Fluorescence Spectroscopies

Roya Mohammadi-Meyabadi [1,2], Negar Beirampour [1], Núria Garrós [1], Helen Lissette Alvarado [1,2], David Limón [2,3], Marcelle Silva-Abreu [1,*], Ana Cristina Calpena [1,2] and Mireia Mallandrich [1,2,*]

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Abstract: The low water solubility of baricitinib (BCT) limits the development of new formulations for the topical delivery of the drug. The aims of this study were to assess the solubility of BCT in different solvents, including Transcutol, a biocompatible permeation enhancer that is miscible in water, to evaluate the drug uptake in human skin and porcine tissues (sclera, cornea, oral, sublingual, and vaginal), and to subsequently extract the drug from the tissues so as to determine the drug recovery using in vitro techniques. Analytical methods were developed and validated for the quantification of BCT in Transcutol using absorption and fluorescence spectroscopies, which are complementary to each other and permit the detection of the drug across a broad range of concentrations. Results show that Transcutol permits an increased drug solubility, and that BCT is able to penetrate the tissues studied. The solutions of BCT in Transcutol were stable for at least one week. Hence, Transcutol may be a suitable solvent for further development of topical formulations.

Keywords: baricitinib; poorly water-soluble drug; solubility study; stability study; in vitro tissue uptake; fluorescence spectroscopy; absorption spectroscopy; drug recovery; drug uptake

check for updates

Citation: Mohammadi-Meyabadi, R.; Beirampour, N.; Garrós, N.; Alvarado, H.L.; Limón, D.; Silva-Abreu, M.; Calpena, A.C.; Mallandrich, M. Assessing the Solubility of Baricitinib and Drug Uptake in Different Tissues Using Absorption and Fluorescence Spectroscopies. *Pharmaceutics* **2022**, *14*, 2714. https://doi.org/10.3390/pharmaceutics14122714

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1. Introduction

Topical delivery is a noninvasive route and an alternative to oral administration. The topical route involves the skin and the nasal, buccal, sublingual, ophthalmic, rectal, and vaginal mucosae. Some patients struggle with oral administration, while in contrast the topical route is easy to administer, which may improve the patients' compliance; some medications administered orally cause digestive side-effects, but the topical route may avoid this inconvenience. Additionally, drug abuse through the topical dosage form is lower than under oral administration. Topical delivery seeks the permeation of drugs through the skin or mucosae [1]. However, drugs face some barriers in penetrating into the tissues, these can include the presence of mucus on the mucosae, low water content, or the existence of the stratum corneum, which is the outermost skin layer and has the main barrier function [2].

Baricitinib (BCT), named 2-[1-ethylsulfonyl-3-[4-(7 H-pyrrolo[2,3-d]pyrimidin-4-yl)pyrazol-1-yl]azetidin-3-yl]acetonitrile according to IUPAC (Figure 1), is an oral selective and reversible Janus-associated kinase (JAK) inhibitor, which modulates the signaling pathway. It has a known anti-inflammatory profile in patients with autoimmune diseases. The BCT mechanism of action consists of inhibiting the signal transduction of IL-6, IL-12, IL-20, IL-22, IL-23, and IFN-γ [3–6]. BCT was approved by the European Medicines Agency (EMA) for the treatment of moderately to severely active rheumatoid arthritis (RA) in adults and for the management of specific cases

of atopic dermatitis [7]. Recently, the US Food and Drug Administration (FDA) approved BCT for emergency use in the treatment of COVID-19 due to its capability of modulating the immunopathology associated with SARS-CoV-2 infection, as well as for the treatment of alopecia areata in adults [8,9].

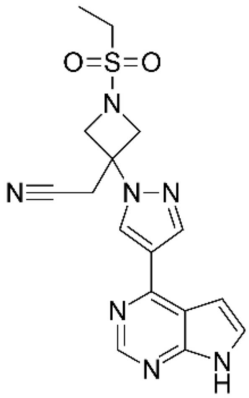

Figure 1. Chemical structure of BCT.

The therapeutic effectiveness and safety of BCT have been investigated, showing sufficient effectiveness and tolerability in clinical trials [10,11]. It is typically administered orally, after which 80% oral bioavailability has been reported in healthy human subjects, but it decreased by 11–18% in the presence of high-fat meals [8]. BCT has a relatively low molecular weight (371.42 Da), and, although BCT is very poorly soluble in water (0.357 mg/mL to 0.46 mg/mL at 25 °C) [3,12], it is bound to plasma and serum proteins; thus, its oral administration implies systemic distribution. After intravenous administration, its volume of distribution is high (76 L), confirming that distribution to the tissues is significant, which is also in line with its low water solubility.

The typical oral doses range from 1 to 4 mg, indicating that BCT is a very potent drug, especially when its high distribution volume is considered [13]. Therefore, oral administration requires sufficiently high doses so as to achieve therapeutic concentrations in the tissues undergoing inflammatory processes. However, at the same time, it implies potential secondary effects.

The achievement of efficacy while decreasing potential secondary effects can be achieved by topical administration, using administration routes such as dermal, ophthalmic, injectable, etc. [6,14,15].

Formulation approaches for the enhancement of the bioavailability of BCT are very scarce in the literature [16,17], most probably due to its very low solubility in water. Moreover, BCT is poorly soluble in ethanol (0.40 mg/mL), but it is freely soluble in organic solvents such as dimethyl sulfoxide (74 to 165.1 mg/mL) and dimethylformamide (50 mg/mL) [12,16]. The toxicity of the organic solvents might have also influenced in the lack of suitable formulations for alternative routes of administration. In contrast, BCT is soluble in PEG-400 (72.4 mg/mL) [12], which is in turn soluble in water. However, PEG-400 is a polymer of nine units of ethylene glycol, and, although it is considered biocompatible, it has been reported that increasing the number of ethylene glycol units of the PEG decreases intestinal permeability [18]. Apart from these solvents, the solubility of BCT in other solvents or its stability in solution have not been studied systematically. Moreover, some analytical methods have been developed to quantify BCT, such as high-performance liquid chromatography (HPLC) and liquid chromatography–mass spectrometry (LCMS/MS). However, despite their advantages such as repeatability, high sensitivity, and reliability, HPLC methods imply a high cost, complex data processing, and greater time consumption. In contrast, UV/Vis absorption spectroscopy or fluorescence spectroscopy which has advantages over other methods, such as its ease and simplicity, fair sensitivity, relatively low cost, and low time consumption [19–21]. Although few analytical methods using

these techniques have been developed for BCT [22], the absorption range of absorption wavelengths of BCT in the UV region implies high interference from other compounds, especially biological components, for which it limits the quantification of BCT from biological samples. Furthermore, fluorescence spectroscopy on the other hand is a suitable and simple, inexpensive, rapid, and reproducible technique used for evaluating fluorescent compounds [23]. Compared to absorption spectroscopy, fluorescence spectroscopy is much more sensitive, permitting much lower limits of detection (LOD) and limits of quantification (LOQ). Because of the low solubility of BCT, the assessment of its in vitro availability in different tissues upon topical administration might require highly sensitive techniques such as this one.

Transcutol, also called diethylene glycol monoethyl ether, is a polymer of three units of ethylene glycol, and it is known to be a biocompatible solvent and permeation enhancer [24]. It is a clear and colorless liquid, which is water-soluble with a melting point of −76 °C [25]. It is widely used in pharmaceutical products, cosmetics, and food because of its low toxicity and high capacity as a solubilizer. Transcutol has been used in oral and sublingual solutions, as well as in injectable products. It has been included in creams, emulsions, gels, ointments, and solutions for topical delivery, covering a broad range of drugs and applications, including analgesic, anti-inflammatory, antifungal, hormones, and veterinary products [26]. Despite these advantages, Transcutol has never been used as a solvent for BCT in any studies. We should take into account that baricitinib presents severe side-effects, and the topical route is an alternative to the oral one, especially when local effects are intended. For instance, patients with atopic dermatitis who do not require systemic immunosuppressant therapy might benefit from topical formulations because this route may avoid systemic side-effects.

The aims of this study were firstly (i) to test the solubility of BCT in different solvents (aqueous solutions, oils, surfactants, and permeation enhancers such as Transcutol). It was to be a preliminary and pre-formulation study before leading to further developing formulations for topical delivery; (ii) to evaluate the uptake of the drug in different tissues (buccal, sublingual, nasal, vaginal, corneal, scleral mucosae, and skin) and the drug recovery; and (iii) to validate the absorption and fluorescence spectroscopy methods. As part of the validation, the stability of BCT solutions in Transcutol was studied at different temperatures.

2. Materials and Methods

2.1. Chemicals and Reagents

The BCT bulk powder ingredient was supplied by Henrikang Biotech Co., Ltd. (Xi'an, China). Transcutol, Labrafac, Isostearyl Isostearate, Labrasol, Lauroglycol 90, Labrafil M 1944 CS, Plurol oleique, and Capryol 90 were acquired from Gattefossé (Saint-Priest, France). Dimethyl sulfoxide (DMSO), Tween-80, and oleic acid were provided by Panreac Química SA (Barcelona, Spain); limonene, α-pinene, nonane, 1-decanol 99%, octanoic acid, lauryl sulfate, sebacic acid, castor oil, and phosphate-buffered solution pH 7.4 were purchased from Sigma Aldrich (St. Louis, MO, USA); Surfadone LP 100, N-ethyl pyrrolidone (NEP), and *N*-methyl pyrrolidone were obtained from ISP (West Yorkshire, UK). Perhydro Squalene was acquired from Fagron Iberica (Terrassa, Spain), liquid paraffin was supplied by Roig Farma, (Terrassa, Spain), and distilled water and purified water were obtained using a Station 9000 purification unit.

2.2. Biological Material

In this work, different tissues were used to assess the drug uptake: buccal, sublingual, vaginal, corneal, and scleral mucosae were of porcine origin, and ex vivo human skin was employed. Porcine specimens were obtained under veterinary supervision from residual individuals of female pigs (cross Landrace × Large White, 25–30 kg) in accordance with the protocol described by Pérez-González and coworkers [27]. In accordance with the 3R rules, the animals had been used previously in surgical university practices, in accordance

with the Ethics Committee of Animals Experimentation at the University of Barcelona. The tissues were immediately transported to the laboratory immersed in artificial aqueous humor solution to be debrided and plain-prepared for the experiments. Human skin was obtained from abdominoplasties practiced on healthy women (Barcelona SCIAS Hospital, Barcelona, Spain). The Bioethics Committee of the Barcelona-SCIAS Hospital approved the study protocol (N°002; dated 17 January 2020). The skin was stored at −20 °C until the experiments were carried out.

2.3. Screening of Solubility in Different Oils and Enhancers

Solubility is a common challenge faced in the development of galenic formulations, as many drugs are poorly water-soluble. Actually, about 40% of new chemical entities are water-insoluble. Taking into account that only a fraction of the drug dissolved will be absorbed at the absorption site, several strategies have been used to enhance the solubility of poorly water-soluble drugs. Before going into any pharmaceutical development, the solubility of BCT was assessed in different solvents, including aqueous media, oils, and other kinds of solutions.

The solubility study was evaluated by the subsequent addition of BCT to 10 mL of solvent, using continuous sonication of each addition at 25 °C for 20 min. Small amounts of BCT (tip of a spatula) were added to the solvent until saturation was observed. The solutions were filtered through a 0.45 µm pore size nylon membrane and analyzed using absorption spectroscopy or fluorescence spectroscopy (see Section 2.5). The following solvents were tested: Transcutol, Labrafac, Isostearyl Isostearate, Lauroglycol 90, Capryol 90, Limonene 97%, *α*-pinene, 1-decanol 99%, lauryl sulfate, sebacic acid, castor oil, Surfadone LP 100, *N*-ethyl pyrrolidone, N-methyl pyrrolidone, liquid paraffin, distilled water, phosphate-buffered solution pH 7.4 (PBS), and mixtures of PBS:Transcutol. Of all the solvents tested, the selection of the solvent for the drug recovery assay (Section 2.4) was based on the solubilization capacity of the solvent also taking into account the biocompatibility of the solvent with the tissues.

2.4. Drug uptake and Drug Recovery from the Skin and Mucosae

In topical delivery, the target may be local or systemic. When the local effect is intended, the target sites may be the tissues beneath the site of application or deeper regions [28]. Thus, it is important to evaluate the capacity of the tissues of interest to uptake a given drug. For this purpose, the recovery of BCT in different tissues was performed. The following tissues were included in this study: human skin, and porcine buccal, sublingual, vaginal, corneal, and scleral mucosae. The skin and some mucosae were cut in horizontal sections using a dermatome (GA630, Aesculap, Tuttlingen, Germany) at 0.4 mm thickness for the skin and at 0.5 mm for the following mucosae: nasal, buccal, and sublingual. This thickness is commonly used in ex vivo permeation assays [29,30]. In the case of the skin, it presents its main representative layers (stratum corneum, viable epidermis, and part of the dermis). The cornea and sclera were isolated from the eyeball and used at full thickness [31], and the same process was followed for the vaginal mucosa [32].

The goal of this assay is to assess the amount of drug able to penetrate the tissue (uptake) and the efficacy of the extraction method (recovery). Stated succinctly, recovery consists of two phases: the uptake process (phase 1), followed by the drug extraction process (phase 2), as summarized in Figure 2.

To perform phase 1, a plain solution of BCT in Transcutol was prepared at a known concentration (C_0). Next, 4 mL of this solution were added to pieces of tissues of 0.64 cm^2, which had been previously weighed. The tissues included in the study were previously evaluated for integrity by means of the measurement of transepidermal water loss (TEWL) or transmucosal water loss (TMWL), and only those tissues that met the criterium were used [33–35]. The vials were placed in a water bath at 32 °C for cornea and skin, and at 37 °C for the remaining tissues, corresponding to the cutaneous and body temperatures, respectively. The incubation time was 24 h for the skin and 6 h for the other tissues. These

periods are common in the duration of the ex vivo permeation assays [29,30,32,36,37]. The C_0 solution was also incubated in the water bath in the same conditions as the tissues so as to evaluate any potential degradation of the drug in the experiment conditions. Control samples of each tissue were also incubated in Transcutol without BCT in the same conditions. The study was conducted in sextuplicate. After the incubation process, the supernatants were collected for further analysis. Absorption spectroscopy (spectrophotometric method) was used to analyze the concentrations of the solutions before incubation (C_0) and after incubation (C_x) solutions [38]. The difference in concentration before and after incubation corresponded to the drug uptake by the tissue, while taking into account the control tissues with Transcutol (the solvent) without the drug. This permitted the evaluation of any interference of the tissue components during the sample analysis [39,40].

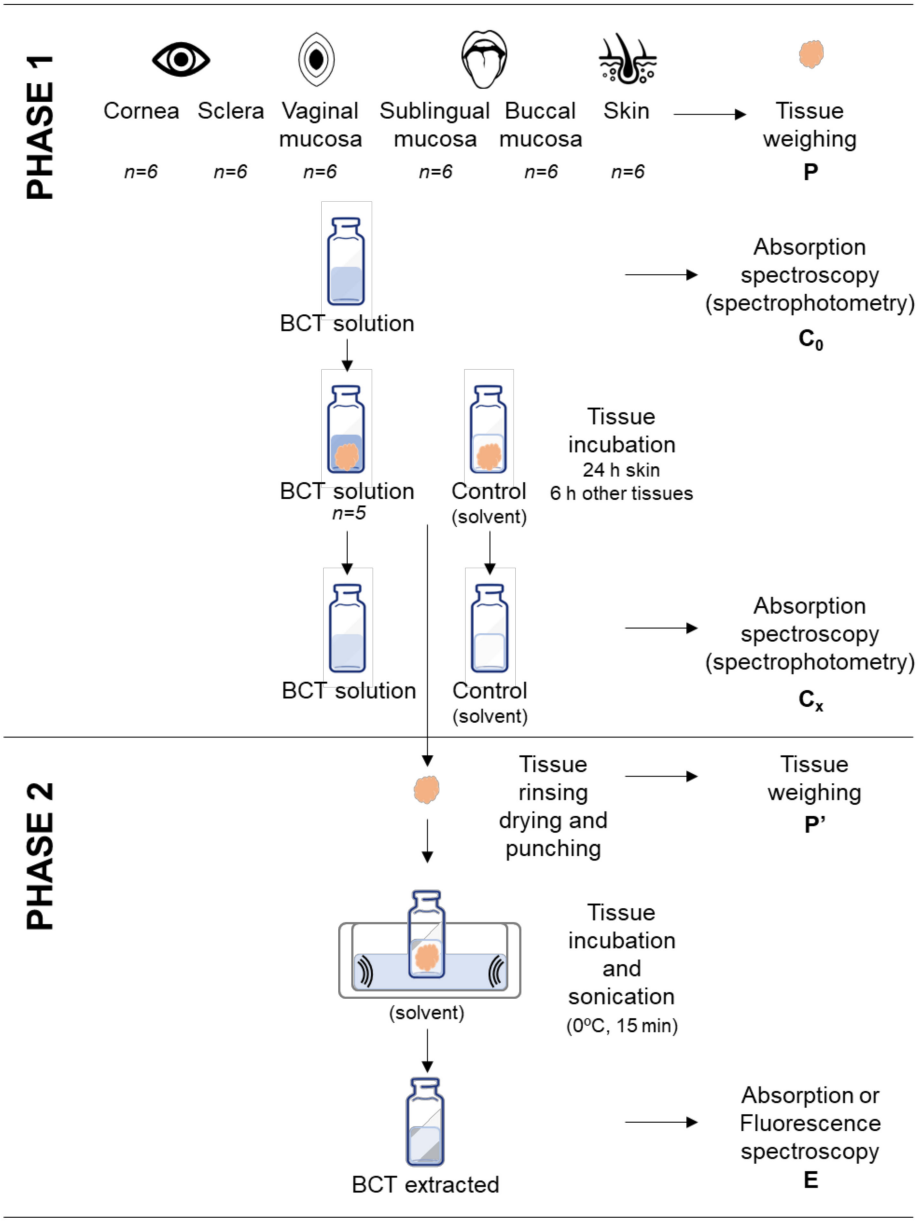

Figure 2. Schematic representation of the recovery process comprising phase 1 (drug uptake) and phase 2 (drug extraction). Samples consisted of pieces of tissue incubated in a solution of baricitinib, and the controls were tissues incubated in the solvent without baricitinib so as to evaluate any interference from the tissue with the analytical methods.

After the incubation for the drug uptake, the tissues were rinsed with purified water, blotted, punched, and weighed again for the subsequent drug extraction (phase 2). Then, 1 mL of Transcutol was used as the extraction solvent, which was added to the vials containing the drug-loaded tissues, and then the vials were sonicated in an ultrasonic water bath for 15 min in ice to prevent thermal degradation. The supernatant of each sample was analyzed by a fluorimeter or spectrophotometer depending on the sample concentration yielding the drug extracted from the tissues. Drug recovery is the relationship between the drug that has penetrated the tissue (uptake) and the drug extracted from the tissue, and it is expressed as a percentage. The percentage recovery (R%) was calculated using Equation (1):

$$R\% = \frac{\frac{E}{P'}}{\frac{C_0 - C_x}{P}} \times 100, \quad (1)$$

where E is the concentration of BCT after extraction process (phase 2), P' is the weight of tissue after extraction, P is the weight of tissue in the uptake phase (phase 1), C_0 is the initial solution at a known concentration of BCT, and C_x is the concentration of the supernatant after the incubation (uptake phase, phase 1).

2.5. Analytical Methods

The wide concentration range of BCT shown in solubility studies and in recovery studies requires the development of two analytical methods which are complimentary to each other, and for which absorption spectroscopy (spectrophotometric method) and fluorescence spectroscopy (fluorometric method) were used. As fluorescence spectroscopy is much more sensitive than absorption spectroscopy, it permits the analysis of BCT concentration in solvents in which it is poorly soluble or in samples from recovery experiments (once the drug has been incorporated and extracted back from the tissue), whereas absorption spectroscopy permits the analysis of higher concentrations of BCT, such as those handled during the uptake into the tissues (see Section 2.4). The solvent used to develop and validate the analytical methods was based on high solubility and tolerability.

2.5.1. Preparation of the Standard Stock Solution

The standard stock solution was prepared by dissolving 20 mg of drug in 20 mL of Transcutol to get the concentration of 1000 µg/mL.

2.5.2. Preparation of the Calibration Curve

All calibration curve solutions were prepared using Transcutol as the solvent. Starting from the stock solution (1000 µg/mL), dilutions were prepared in the following concentrations for absorption spectroscopy: 60, 50, 40, 30, 25, 20, 15, 10, 7.5, and 6.25 µg/mL, whereas the following concentrations were established for fluorescence spectroscopy: 1.25, 0.625, 0.312, 0.156, and 0.078 µg/mL.

2.5.3. Absorption Spectroscopy (Spectrophotometric Method)

Absorbance measurements were performed using a PerkinElmer UV/Vis spectrophotometer (Shelton, CT, USA) and a 1 cm quartz cuvette. First, a spectrum was acquired in the wavelength range 250 nm to 400 nm. The maximum absorption was observed at 310 nm, for which absorbance values were read at this wavelength. A control experiment was carried out simultaneously in Transcutol (without BCT).

2.5.4. Fluorescence Spectroscopy (Fluorometric Method)

The excitation and emission spectra were obtained using a RF-1501 Fluorimeter (Shimadzu, Canada) (light source = 150 W xenon lamp in self-contained lamp housing), managed by FL Solutions software and using a quartz cuvette. On the basis of the results, the fluorometric conditions to quantify the concentrations of BCT on all samples were

an excitation wavelength of 310 nm and an emission wavelength of 390 nm. A control experiment was carried out simultaneously in Transcutol (without BCT).

2.6. Validation of the Analytical Methods

Validation concerned the evaluation of linearity, range, accuracy, and precision in accordance with the standard rules of the European Medicines Agency [41] and *Asociación Española de Farmacéuticos de la Industria* [42]. The limits of detection and quantification of the methods were also determined. The stability of the standard solutions was also evaluated.

2.6.1. Linearity and Range

Linearity is the ability within a defined range to obtain results directly proportional to the concentrations of the analyte in the sample [41]. The range is the interval defined by the upper and lower concentrations of the tested drug for which it has been proven that the method has a suitable level of accuracy, precision, and linearity [41]. Linearity was assessed with five calibration curves at 10 concentration levels for the spectrophotometric method and five concentration levels for the fluorometric method. Individual slopes between the instrumental signals versus the corresponding drug concentrations were calculated. The least-squares regression was calculated using Equation (2), reporting the corresponding determination coefficients (r^2).

$$Abs = S{\cdot}C + a, \tag{2}$$

where C is the concentration, Abs is the absorbance in the spectrophotometric method or the emission in the fluorometric method, S is the value of the slope, and a is the y-intercept. One-way analysis of variance (ANOVA) was performed to compare the signals versus nominal concentration at each concentration searching for nonsignificant differences (significance level set at $p < 0.05$). Results were processed using the Prism®, V.5.00 software (GraphPad Software Inc., San Diego, CA, USA).

2.6.2. Sensitivity

The limit of detection (LOD) is the lowest amount of analyte in the sample that can be detected by the method, and the limit of quantification (LOQ) is the lowest amount of analyte in the sample that can be determined by precision, accuracy, and linearity. The LOD and LOQ were calculated according to the standard deviation of the response and the slope of the calibration curve using Equation (3):

$$LOD\ or\ LOQ = \frac{K \times SD_a}{S}, \tag{3}$$

where K is a factor related to the level of confidence (3.3 for LOD and 10 for LOQ). SDa is the standard deviation of the intercept (a), and S is the slope of the calibration line.

2.6.3. Accuracy and Precision

The accuracy of an analytical method indicates the capacity of the method to provide results close to the real value. In this study, the accuracy was evaluated using standard solutions of five calibration curves: within the concentration range of 6.25 to 60 µg/mL for spectrophotometry and within 0.078 to 1.25 µg/mL for fluorometry. The accuracy at each concentration level was expressed as the mean percentage deviation or relative error (RE%) with respect to the nominal concentration, calculated using Equation (4).

$$\%RE = \frac{C_{obs} - C_{nom}}{C_{nom}} \times 100, \tag{4}$$

where C_{obs} is the observed concentration, and C_{nom} is the nominal concentration of each standard solution.

Precision is the degree of variance between measurements. To assess the precision of the method, the standard solutions were prepared by two different analysts on two different

days (intermediate precision), and it was expressed as the relative standard deviation (RSD%) of the different replicates at the different concentration levels, using Equation (5).

$$\%RSD = \frac{SD}{C_{nom}} \times 100, \quad (5)$$

where SD is the standard deviation of the replicates at a certain concentration, and C_{nom} is the nominal concentration.

2.6.4. Stability

The stability of standard solutions was evaluated at two levels, the lowest and the highest concentration of the calibration curves of both methods, i.e., 6.25 µg/mL and 60 µg/mL for the spectrophotometric method, and 0.078 µg/mL and 1.25 µg/mL for the fluorometric method. The four solutions were incubated at three different temperatures: room (22 ± 2 °C), refrigerator (5 ± 3 °C), and freezer (−20 ± 5 °C).

The standard solutions were analyzed at time 0 (freshly prepared), on day 1, and on day 7. The stability was calculated as the relative difference between the absorbances at each stability point and the absorbance of the freshly prepared solution, as shown in Equation (6).

$$Relative\ difference\ (\%) = \frac{Abs_t - Abs_{t0}}{Abs_{t0}} \times 100, \quad (6)$$

where Abs_t is the absorbance of the standard solution at the stability point, and Abs_{t0} is the absorbance of the standard solution just prepared.

3. Results and Discussion

3.1. Solubility Studies in Different Oils and Enhancers

Solubility is one of the most important physicochemical attributes for drug development, as low solubility can hinder the development of different products and severely limit the bioavailability of orally administered dosage forms [43]. Moreover, low aqueous solubility (<1 mg/mL) [44] is the main problem encountered in the development of formulations of new chemical entities [45,46]. The estimation of the solubility using the solid dispersion method for a given drug in different solvents and cosolvents is important for the development of future formulations which can increase the bioavailability of the drug of interest [12,47,48]. In this study, the solubility of BCT was evaluated in the aqueous solvents reported in Table 1. To this end, the saturated solutions prepared in the different solvents (see Section 2.3) were filtered and analyzed by absorption spectroscopy or fluorescence spectroscopy using the validated methods. According to the range of concentrations in which the analytical methods were developed and validated, Tables 1 and 2 show the solubilities estimated using absorption spectroscopy, whereas Table 3 shows those estimated using fluorescence spectroscopy.

The solubility of BCT was tested in pure water, phosphate-buffered saline at pH 7.4, and mixtures of both solvents. Despite being a poorly water-soluble drug (<1 mg/mL) [44], the European Medicines Agency classifies it as Class III in the Biopharmaceutical Classification System (BCS) [49], i.e., poorly permeable and highly soluble [16]. This discrepancy in the classification of baricitinib as poorly or highly water-soluble is due to the different criteria used to classify the drug's solubility. While the USP indicates that a drug is poorly soluble when its solubility is below 1 mg/mL [44], the grade of solubility in the BCS is defined as highly soluble when "the highest dose strength is soluble in less than 250 mL water over a pH range of 1 to 7.5" [50]. Considering that the highest strength of baricitinib is 4 mg, the resulting concentration in 250 mL of water is much lower than 1 mg/mL (i.e., soluble). However, according to the BCS criteria, the drug is highly soluble. The addition of Transcutol to PBS increases the amount of BCT that the medium is able to dissolve. A low proportion of Transcutol slightly improved the solubility profile of BCT in PBS, and better results were observed when the proportion of Transcutol is 50%, which increased the solubility of BCT more than 100 fold.

Table 1. Solubility of BCT in aqueous solutions tested by absorption spectroscopy.

Solvent	Solubility (μg/mL)
PBS 7.4: Transcutol (1:1 *v*/*v*)	2033.71 ± 0.74
PBS 7.4: Transcutol (95:5 *v*/*v*)	25.21 ± 0.67
PBS pH 7.4	16.18 ± 0.74
Water	10.86 ± 0.67

PBS: phosphate buffered saline.

Table 2. Molecular weight, lipophilia, and estimated solubility of BCT in different oils and enhancers by absorption spectroscopy. Results are reported from the highest to the lower solubility of BCT in the solvents.

Popular Name	Chemical Name (IUPAC)	Molecular Weight (g/mol)	Lipophilia (Log P)	Solubility (μg/mL)
N-Ethyl pyrrolidone	1-Ethylpyrrolidin-2-one	113.16	−0.04	69518 ± 1390
Transcutol	Diethylen glycol monoethyl ether	134.17	−0.54	10817 ± 325
Capryol 90	2-Hydroxypropyl octanoate	202.29		6513 ± 261
Lauroglycol 90	2-Hydroxypropyl dodecanoate	258.4	1.14	600.19 ± 24.01
Labrafac	A mixture of medium-chain triglycerides mainly from caprylic (C8) and capric (C10) acids	NA	NA	503.74 ± 25.19
Surfadone LP-100	1-Octylpyrrolidin-2-one	197.32	NA	93.83 ± 4.69
1-Decanol	Decan-1-ol	158.28	4.57	90.74 ± 5.44
Isostearyl isostearate	16-Methylheptadecyl 16-methylheptadecanoate	537	NA	55.76 ± 3.90
Azone	2-Hydroxypropyl octanoate	202.29	NA	53.52 ± 4.28
Sebacic acid	Decanedioic acid	202.25	2.20	49.24 ± 4.43
Lauryl sulfate	Dodecyl hydrogen sulfate	266.4	NA	23.07 ± 0.31
α-Pinene	2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	136.23	4.83	3.79 ± 0.09

NA: Not available.

Table 3. Solubility of BCT in the oils and enhancers tested using fluorescence spectroscopy. Results are reported from the highest to the lower solubility of BCT in the solvents.

Popular Name	Chemical Name (IUPAC)	Molecular Weight (g/mol)	Lipophilia (Log P)	Solubility (μg/mL)
Paraffin	Paraffinum liquidum	436.84	NA	0.29 ± 0.01
Castor oil	2,3-Bis[[(Z)-12-hydroxyoctadec-9-enoyl]oxy] propyl (Z)-12-hydroxyoctadec-9-enoate	933.4	NA	0.20 ± 0.01
N-Methyl pyrrolidone	1-Methyl-2-pyrrolidinone	99.13	−0.38	0.08 ± 0.00
Limonene 97%	(4R)-1-Methyl-4-prop-1-en-2-ylcyclohexene	136.23	4.57	0.01 ± 0.00

NA: not available.

The greatest solubility was found to be in N-ethyl pyrrolidone, followed by Transcutol (Table 2).

An effective strategy to enhance drug permeation is to increase the concentration of drug dissolved because the rate of permeation (flux) increases proportionally to the drug concentration due to an increase in the thermodynamic activity together with the partition of the drug [1,51]. Bolla and coworkers investigated the solubility of ibuprofen in different solvents including Transcutol, propylene glycol, and isopropyl myristate. The greatest solubility was observed in Transcutol and propylene glycol. The researchers evaluated the permeability in vitro of ibuprofen formulated in different dosage forms (creams, hydrogels, and nonaqueous gels), and they observed that the nonaqueous gels containing Transcutol and propylene glycol exhibited higher amounts of drug permeated per unit area [51]. Hence, the solvent for developing topical formulations must be selected very carefully as it may have a great impact on drug permeability.

Some compounds are able to promote the rate and extent of drugs permeated into the tissues by disrupting the barrier function of the tissues. These are defined as penetration

enhancers and are added to the formulations to increase or modulate the permeation of drugs. The penetration enhancers should be nontoxic, nonirritant, and non-sensitizing, with nonpharmacological activity, and they should exhibit a rapid onset of action in reducing the tissue barrier resistance while maintaining a reversible effect, whereby the skin or mucosa can recover their properties. Moreover, penetration enhancers should also be compatible with the formulation.

Transcutol is considered nontoxic and biocompatible with the skin and other tissues. It is listed in the Food and Drug Administration in the United States at 5% for transdermal use and at 25% for topical use. Health Canada approved Transcutol as a natural health product, and in 2002, the Cosmetic Ingredient Review expert panel evaluated 622 products containing Transcutol from 0.0004% to 80% and concluded that Transcutol is safe for use in cosmetics [26]. Gad et al. investigated the toxicity of Transcutol in different animal models and routes of administration; for instance, Transcutol was well tolerated in cats when administered intravenously at 2 mL/kg. The researchers also evaluated the tolerability of 50% Transcutol applied dermally in rabbits on a skin surface area of 2 cm^2 with no signs of skin irritation. The acute toxicity study was conducted in rats administering 5 g/kg orally; the lethal dose 50 (LD 50) was established above 5000 mg/kg [52]. Considering Transcutol's capacity in solubilizing BCT and its safety profile, it was selected as the vehicle for the subsequent study of drug uptake by different tissues and drug recovery from the tissues (Section 3.2).

3.2. Drug Uptake by the Tissues and Recovery from the Skin and Mucosae

The amount of BCT available in different ex vivo tissues was studied by carrying out recovery experiments. Firstly, the tissues were incubated in a solution of BCT in Transcutol at a known concentration for 6 or 24 h at 32 or 37 °C (drug uptake phase—phase 1), where the amount of BCT that can penetrate and be retained within different tissues, and thus the amount of BCT that can be extracted from the tissues (recovery phase—phase 2) were determined. This methodology is commonly used in dermal and transdermal delivery to determine the percentage recovery of the drug using a specific extraction method. The recovery allows the calculation of the amount of drug retained in the skin from the extraction data by multiplying the result by the recovery factor. Silva-Abreu and coworkers studied the permeation of pioglitazone loaded in polymeric nanoparticles in ex vivo mucosae, including buccal, sublingual, nasal, and intestinal from porcine origin. After the permeation study, the researchers assessed the amount of pioglitazone uptake by the mucosae by extracting the drug with a solvent under an ultrasonic bath treatment and subsequent correction by the recovery factor [53]. Gómez-Segura et al. used the same technique to determine the amount of carprofen uptake by ex vivo conjunctiva, cornea, sclera, buccal, sublingual, and vaginal mucosae [54]. This technique has also been applied to in vivo studies; Miralles et al. introduced pioglitazone nanoparticles into the eyes of pigs and analyzed the samples from the aqueous humor, vitreous humor, cornea, sclera, and lens after a drug extraction procedure. The study revealed that the drug was mainly located in the sclera [55].

In this work, results show that similar amounts of BCT per gram of tissue could penetrate the different tissues studied, except for the buccal mucosa, which could incorporate the greatest amount of BCT (208.68 µg/g). From the BCT incorporated within the tissue, the extraction procedure was able to recover 27.52% for the cornea, 25.01% for the sublingual, and 16.44% for buccal mucosae. Recoveries below 10% were found for the remaining tissues (Table 4).

The differences in the drug amount that penetrates the tissues may be due to structural differences between the tissues. The skin and the cornea are stratified, and each layer differs in water, lipid, and protein content [12]. The skin is the largest organ of the body; it represents about 15% of the body weight with a surface area of about 2 m^2 [1] and an average thickness of 2–3 mm depending on the age and anatomical site. Additionally, the skin's outermost layer (the stratum corneum) is a keratinized structure, which is the

main barrier to drug permeation [56] with pH ranging 4.5–6 [57]. The low penetration in the skin may be due to the stratum corneum which hinders drug permeation. The oral cavity has a surface area of about 200 cm^2, of which 50 cm^2 correspond to the buccal mucosa and 25 cm^2 to the sublingual mucosa. The nasal mucosa covers about 130 cm^2. The pH of these mucosae ranges from 5.0–7.5 approximately, with a thickness of 0.7–1 mm for the nasal, 0.5–0.8 mm for the buccal, and 0.1–0.2 mm for the sublingual mucosae. Concerning the water content, the fluid volume on all three mucosae was 0.1–1 mL [2]. The administration of drugs via the vaginal route has been extensively used, either for local therapy to manage different infections and spermicidal agents, or for systemic therapy such as hormone supply [58]. The vaginal epithelium is stratified and non-keratinized with a thickness of 0.2–0.5 mm. The vaginal fluid (including the vaginal mucus) covers the epithelium to protect the vagina against pathogen infections, while acting as a barrier to drug penetration [59].

Table 4. Drug uptake (determined by the amount of drug that has penetrated the tissue) and recovery of BCT in the tissues tested. The sample analysis was carried out by spectrophotometry and/or fluorimetry depending on the sample concentration.

	Penetration (μg/g) [a]	Recovery (μg/g) [a]	Recovery (%)
Skin	38.79 ± 4.27	1.79 ± 0.19	4.61 ± 4.49
Buccal	208.68 ± 25.04	34.31 ± 4.12	16.44 ± 1.97
Sublingual	20.05 ± 2.60	5.02 ± 0.65	25.01 ± 3.25
Vaginal	61.54 ± 8.62	2.22 ± 0.33	3.61 ± 0.54
Cornea	48.12 ± 7.70	13.24 ± 2.12	27.52 ± 4.40
Sclera	27.03 ± 4.59	2.02 ± 0.34	7.46 ± 1.27

[a] μg of BCT per g of tissue.

Ocular drug delivery faces physiological and anatomical barriers, tears with a volume of about 7 μL clear more than 95% of the applied dose in less than half a minute after instillation. The cornea is a transparent tissue of about 500 μm thickness [60] and offers significant resistance to drug permeation because of the tight junctions between epithelial cells, in addition to its lipidic nature. The sclera is composed of collagen fibers and proteoglycans with a thickness of about 300 μm [60]. The permeability of the drugs is affected by the size and charge of the molecules, being inversely proportional to the size and resulting in lower permeability of those molecules with positive charge [61].

The mucus covers the mucosal surface including the gastrointestinal tract, vagina, and eyes. In addition to lubricating and hydrating the epithelia, it acts as a barrier to drug penetration. It is mainly composed of mucins and water. The thickness of the mucus differs in the different mucosae; it is approximately 10 μm for the nasal mucosa, 85 μm for the buccal and sublingual mucosae, 50 μm for the vaginal mucosa, and between 3 to 30 μm on the eye [2,62,63].

The characteristics and particularities of the tissues and the existing barriers limit the bioavailability of the drug; hence, strategies to increase drug solubility and to overcome the skin and mucosal barriers are often required in topical delivery, and which include the use of physical or chemical enhancers, micro and nanoemulsions, nanoparticles, nanocrystals, lipid-based formulations, micelle-based formulations, etc. [64].

Another point to take into account is that the permeation of a given drug depends on the drug's physicochemical properties, as well as on the partition coefficient formulation/tissue, which is indicative of the affinity of the drug for the vehicle in which it is formulated and for its affinity to the tissue. It is possible to elucidate the mechanism involved in the permeation of the drug by calculating the diffusion and partition coefficients after ex vivo permeation tests. Mallandrich and coworkers evaluated the permeation of ketorolac loaded in polymeric nanoparticles through corneal and scleral tissues. The researchers found that the diffusion coefficient had similar values in the cornea and sclera,

and the effect of the partition coefficient was greater than that of the diffusion coefficient, and this parameter differed between the cornea and the sclera, indicating that the tissue has an influence on the permeation of the drug [32]. In this study, the uptake and recovery studies were conducted with the same drug in the same vehicle but in different tissues. Then, further investigation by the ex vivo permeation test using the tissues involved in this study allowed the determining of the partition and diffusion coefficients and shed light on the impact of the tissue on drug permeation [1].

Another aspect to consider is that the tissue pieces were immersed in the solution of baricitinib, and the drug probably penetrated the tissues through the surface, as well as through the inner part of the tissue and via a lateral entrance. In an in vivo application, only the surface of tissue is in contact with the solution; since the permeation of the drug is surface-dependent, the drug uptake may be higher under these conditions than in real applications. Nevertheless, the goal of the recovery study was not to evaluate the extent of permeation itself, but to determine the efficacy of the extraction method so as to obtain the correction factor for calculating the drug remaining in the skin after ex vivo permeation tests, thus informing of the rate and extent of permeation for each tissue.

3.3. Analytical Methods Validation

So as to develop appropriate delivery systems for topical routes of administration, adequate solvents must be first found. They must be biocompatible and they must permit a sufficient amount of dissolved drug. Then, analytical methods must be developed and validated for the quantification of the drug in different samples.

Due to its low water solubility, BCT was added and dissolved in different oils and solutions (10 mL) using continuous sonication until saturation was observed. The following solvents were tested: Transcutol, Labrafac, Isostearyl Isostearate, Lauroglycol 90, Capryol 90, limonene 97%, α-pinene, 1-decanol 99%, lauryl sulfate, sebacic acid, and castor oil, Surfadone LP 100, *N*-ethyl pyrrolidone, *N*-methyl pyrrolidone, and liquid paraffin.

Of the solvents tested, *N*-ethyl pyrrolidone was the solvent which showed the highest amount of BCT dissolved, followed by Transcutol. However, *N*-methyl pyrrolidone shows high cytotoxicity, whereas Transcutol is highly biocompatible [65,66]. For this reason, Transcutol was selected for further recovery experiments in different tissues and, consequently, also used as the solvent to develop the analytical methods.

Absorption spectroscopy of BCT in Transcutol shows a strong absorption at 310 nm (UV range) corresponding to BCT (Figure 3A), which is in accordance with the extended pi conjugation in the molecule (Figure 1).

Similarly, fluorescence spectroscopy showed a maximum excitation at 310 nm (Figure 3B) and a maximum emission at 388 nm (Figure 3C).

3.4. Validation of the Analytical Methods

Calibration curves were prepared from a stock solution (1000 µg/mL) of BCT in Transcutol, from which dilutions were also prepared using Transcutol and analyzed using absorption spectroscopy or fluorescence spectroscopy, in both cases using excitation at λ 310 nm.

For each technique used, five replicates were analyzed, in accordance with the validation guidelines by European Medicines Agency Guideline and Asociación Española de Farmacéuticos de la Industria which indicated that between three to six replicates for each validation should be included [41,42].

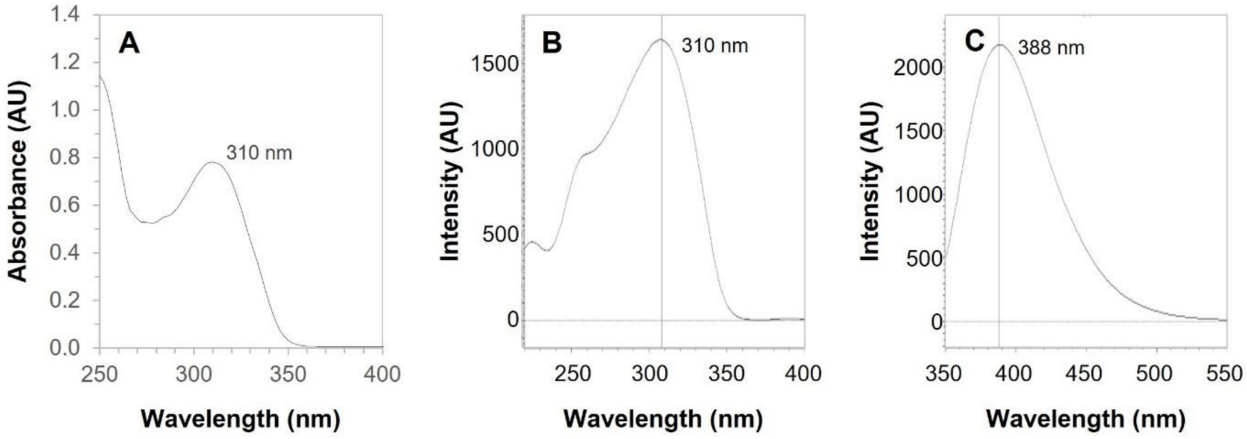

Figure 3. (**A**) Absorption spectrum of BCT in Transcutol. (**B**) Excitation spectrum of BCT in Transcutol. (**C**) Emission spectrum of BCT in Transcutol.

3.4.1. Linearity and Range

Figure 4A shows the calibration curves of BCT in Transcutol using absorption spectroscopy. The linearity of the calibration curves was assessed according to the correlation coefficient. All values of r^2 were above 0.9994. Furthermore, Figure 4B shows a constant response factor (ratio absorbance/concentration) in the concentration range of 6.52 µg/mL to 60 µg/mL, with only slighter variation at the lowest concentrations. Table 5 shows the response factors obtained using absorption spectroscopy, whereas Table 6 shows the response factors using fluorescence spectroscopy. No statistical differences (statistical significance set at $p < 0.05$) were found after ANOVA analysis of the response factors using either technique.

Table 5. Linearity of the spectrophotometric method using ANOVA test for the response factor of the calibration curves.

	R1	R2	R3	R4	R5
Conc	**Abs/Conc**	**Abs/Conc**	**Abs/Conc**	**Abs/Conc**	**Abs/Conc**
6.25	0.03	0.03	0.03	0.03	0.04
7.50	0.03	0.03	0.03	0.04	0.03
10.00	0.03	0.03	0.03	0.04	0.03
15.00	0.03	0.03	0.03	0.03	0.03
20.00	0.03	0.03	0.03	0.03	0.03
25.00	0.03	0.03	0.03	0.03	0.03
30.00	0.03	0.03	0.03	0.03	0.03
40.00	0.03	0.03	0.03	0.03	0.03
50.00	0.03	0.03	0.03	0.03	0.03
60.00	0.03	0.03	0.03	0.03	0.03

Table 6. Linearity of the fluorometric method by ANOVA test of the response factor of the calibration curves.

	R1	R2	R3	R4	R5
Conc	**Int/Conc**	**Int/Conc**	**Int/Conc**	**Int/Conc**	**Int/Conc**
0.078	784.3	790.8	795.4	784.3	789.4
0.156	783.1	784.6	797.2	792.6	784.4
0.313	791.0	794.8	797.2	788.2	782.4
0.625	782.8	777.6	777.6	787.4	798.2
1.250	781.0	780.1	785.9	790.1	784.0

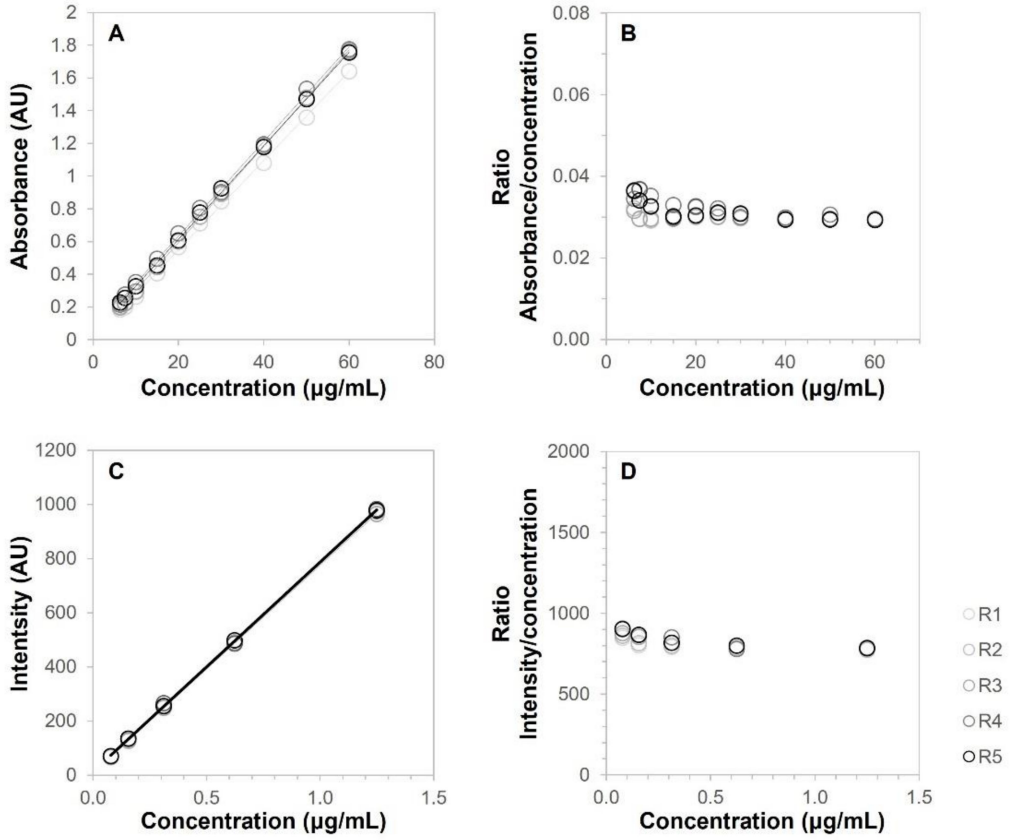

Figure 4. (**A**) Calibration curves of BCT in Transcutol (n = 5) using absorption spectroscopy. (**B**) Response factor (ratio absorbance/concentration) using absorption spectroscopy. (**C**) Calibration curves of BCT in Transcutol (n = 5) using fluorescence spectroscopy. (**D**) Response factor (ratio intensity/concentration) using fluorescence spectroscopy.

The response factor is defined as the ratio between the response of the detector (signal) and the concentration of the analyte. This parameter correlates the signal to the concentration. When a method is linear, the signal increases proportionally to the analyte concentration; thus, the response factor remains constant for the different concentration levels. An ANOVA test of the response factor showed that there were no statistical differences in response factors over the analyte concentrations and confirmed the linearity of both methods, within the studied ranges.

Similarly, Figure 4C shows the calibration curves of BCT in Transcutol using fluorescence spectroscopy, where the correlation coefficient of all replicates was $\geq$0.9998, showing a constant response factor (ratio intensity/concentration) in the concentration range of 0.078 µg/mL to 1.250 µg/mL (Figure 4D). Table 5 shows the response factors obtained, where no statistical differences ($p > 0.05$) were found between replicates after ANOVA analysis.

3.4.2. Sensitivity

According to the standard deviation of the response and the slope of the calibration curves, the LOQ was 2.61 ± 1.21 µg/mL and the LOD was 0.86 ± 0.40 µg/mL using absorption spectroscopy. In contrast, fluorescence spectroscopy led to values two orders of magnitude lower, with an LOD of 0.01 ± 0.00 and LOQ of 0.02 ± 0.01 µg/mL (mean ± SD). Although the absorption spectroscopy presented a higher LOQ, the method was sensitive enough sensitivity to quantify the samples of the uptake phase in the recovery study and most of the samples in the solubility study. For those samples with a low concentration of drug, such as some solvents in the solubility study and samples from the extraction phase

in the recovery study, a more sensitive method was required, which was provided by the fluorescence spectroscopy.

3.4.3. Accuracy and Precision

Accuracy and precision were calculated from the mean of the back-calculated concentrations of calibration curves in both methods, spectrophotometry (Table 7) and fluorimetry (Table 8). Accuracy was expressed as the relative error (%RE) with respect to the nominal concentration, whereas precision was expressed as the relative standard deviation (%RSD) of the predicted concentrations.

Table 7. Accuracy and intermediate precision of the spectrophotometric method.

Theoretical Concentration (μg/mL)	Mean Experimental Concentration (μg/mL)	SD (μg/mL)	RE (%)	RSD (%)
6.25	6.276	0.449	−0.42	7.16
7.5	7.304	0.190	2.61	2.61
10	9.770	0.299	2.30	3.06
15	14.740	0.301	1.73	2.04
20	20.220	0.319	−1.10	1.58
25	25.604	0.285	−2.41	1.11
30	30.219	0.665	−0.73	2.20
40	39.715	0.344	0.71	0.87
50	50.123	0.540	−0.25	1.08
60	59.778	0.208	0.37	0.35
Mean (%)	-	-	−0.28	2.21
SD	-	-	1.59	1.93

SD: standard deviation; RE: relative error; RSD: relative standard deviation.

Table 8. Accuracy and intermediate precision of the fluorometric method.

Theoretical Concentration (μg/mL)	Mean (μg/mL)	SD (μg/mL)	RE (%)	RSD (%)
0.078	0.075	0.001	−3.34	1.97
0.156	0.156	0.002	0.31	1.38
0.312	0.316	0.005	1.34	1.67
0.624	0.622	0.008	−0.36	1.28
1.248	1.248	0.003	0.01	0.21
Mean (%)	-	-	−0.41	1.30
SD	-	-	1.76	0.67

SD: standard deviation; RE: relative error; RSD: relative standard deviation.

For the spectrophotometric method, the relative error obtained was −0.28% ± 1.59%, whereas the relative standard deviation was 2.21% ± 1.93%. Similarly, using the fluorometric method, the relative error was −0.41% ± 1.76%, whereas the relative standard deviation was 1.30% ± 0.67%. In all cases, the values found were much lower than the 15% limit value in accordance with EMA guidelines [41]. Therefore, the method was considered accurate and precise within the concentration range 6.25–60 μg/mL for the spectrophotometric method and within the concentration range 0.078–1.248 μg/mL for the fluorometric method.

In addition to being accurate, precise, linear, and sensitive enough, an analytical method should also be specific for the analyte. Since changes in the matrix of the samples may affect the results obtained, to ensure that the results of the drug uptake and drug recovery were not affected by the tissues, controls were used in the analysis. The controls were pieces of the tissues (sclera, corneal, sublingual, buccal, and skin) incubated in Transcutol (the solvent) without the drug. The tissues underwent the same procedure and conditions as the samples, and, after the incubation and extraction process, the supernatant

was analyzed by spectrophotometry or fluorimetry to evaluate any signal in the analysis. The absorbance/intensity of the controls was subtracted from the values obtained in the samples with baricitinib; doing so, any signal that may have come from the tissue was countered, yielding the drug's concentration within the tissue.

3.4.4. Stability

The stability of the BCT solution in Transcutol was evaluated at two different concentrations, 60 µg/mL (Table 9) and 6.25 µg/mL (Table 10), using the spectrophotometric method and at two different concentrations, 1.25 µg/mL (Table 11) and 0.078 µg/mL (Table 12), using the fluorometric method. The solutions were analyzed freshly prepared and after storage in different conditions: room temperature (22 ± 2 °C), refrigerator (5 ± 3 °C), or freezer (−20 ± 5 °C). The spectrophotometric method was used to analyze the stability of the two highest concentrations, whereas the fluorometric method was used for the two lowest concentrations. The relative difference of the solutions stored at different conditions was below 2% for both methods, leading to the conclusion that BCT is stable in Transcutol for at least 7 days; thus, the samples could be safely stored at 22 °C, 5 °C, or −20 °C for 1 week.

Table 9. Stability of the standard solution 60 µg/mL stored at three different temperatures: room temperature, fridge, and freezer, as analyzed by absorption spectroscopy (spectrophotometry).

	Room Temperature		Fridge		Freezer	
Stability Time (Day)	**Absorbance (AU)**	**Relative Difference (%)**	**Absorbance (AU)**	**Relative Difference (%)**	**Absorbance (AU)**	**Relative Difference (%)**
0 day	1.7628	N/A	1.7643	N/A	1.7535	N/A
1 day	1.7222	−2.30%	1.7608	−0.20%	1.7545	0.06%
7 days	1.7325	−1.72%	1.7297	−1.96%	1.7521	−0.08%

Table 10. Stability of the standard solution of 6.25 µg/mL stored at different temperatures: room temperature, fridge, and freezer, as analyzed by absorption spectroscopy (spectrophotometry).

	Room Temperature		Fridge		Freezer	
Stability Time (Day)	**Absorbance (AU)**	**Relative Difference (%)**	**Absorbance (AU)**	**Relative Difference (%)**	**Absorbance (AU)**	**Relative Difference (%)**
0 day	0.1899	N/A	0.1969	N/A	0.227	0.23
1 day	0.1897	−0.11%	0.191	−3.00%	1.7545	0.57%
7 days	0.1871	−1.47%	0.1985	0.81%	1.7521	−0.88 %

Table 11. Stability of the standard solution 1.25 µg/mL stored at the different temperatures: room temperature, fridge, and freezer, as analyzed by fluorescence spectroscopy (fluorimetry).

	Room Temperature		Fridge		Freezer	
Stability Time (Day)	**Intensity (AU)**	**Relative Difference (%)**	**Intensity (AU)**	**Relative Difference (%)**	**Intensity (AU)**	**Relative Difference (%)**
0 day	975.23	N/A	982.4	N/A	982.44	N/A
1 day	974.26	−0.10%	971.62	−1.10%	976.25	−0.63%
7 days	975.16	−0.01%	982.42	0.00%	976.25	−0.63%

The main limitations of this work are that not all the solvents are suitable as media for the spectrophotometer and fluorimeter. The following solvents were also tested but showed high absorbance values by themselves at the baricitinib wavelength and, thus, were ruled out of the solubility study: polyethylene glycol 400, Tween-80, castor oil, Labrafil, Plurol Oleique, 1-decanol, octanoic acid, and oleic acid.

Table 12. Stability of the standard solution 0.078 μg/mL stored at three different temperatures: room temperature, fridge, and freezer, as analyzed by fluorescence spectroscopy (fluorimetry).

	Room Temperature		Fridge		Freezer	
Stability Time (day)	**Intensity (AU)**	**Relative Difference (%)**	**Intensity (AU)**	**Relative Difference (%)**	**Intensity (AU)**	**Relative Difference (%)**
0 day	61.27	N/A	62.24	N/A	61.24	N/A
1 day	61.18	−0.15%	61.27	−1.56%	61.24	0.00%
7 days	61.79	0.85%	62.03	−0.34%	61.25	0.02%

In other words, although the spectrophotometer and fluorimeter are time-saving and inexpensive techniques with regard to chromatographic methods, some solvents interfere with the analysis and are not suitable.

4. Conclusions

As BCT shows low solubility and low permeability, the solubility of this drug was estimated in different solvents, with the purpose of finding those which are suitable for the future development of formulations which permit increasing its availability in tissues. Among the different solvents tested, Transcutol permitted its dissolution at concentrations up to 10.8 mg/mL, and it was selected because of its reported biocompatibility and low toxicity in the literature.

Moreover, the amount of BCT uptake by the different tissues was determined (human skin and porcine mucosae: buccal, sublingual, and vaginal mucosae, cornea, and sclera). The amount of BCT that could be extracted back out of the tissues was also investigated, leading to the percentage of drug recovery for each tissue.

To conduct the sample analysis in this study, two simple, fast, and inexpensive methods were developed and validated for the analysis of BCT in Transcutol, using either absorption spectroscopy (spectrophotometric method) or fluorescence spectroscopy (fluorometric method). Both methods were shown to be linear, precise, and accurate, and they are complimentary to each other, where absorption spectroscopy met these characteristics at higher concentrations, in the range of 6.25 to 60 μg/mL, whereas fluorescence spectroscopy met them at lower concentrations, in the range 0.078 to 1.25 μg/mL. Therefore, both analytical methods are cost and time-effective and are linear, precise and accurate for the analysis of BCT in samples of different tissues. The choice of the method only depends on the concentration of BCT in samples, where the higher sensitivity of fluorescence spectroscopy permits the analysis of much lower concentrations. Solutions of BCT in Transcutol in the whole range of concentrations are stable for up to 7 days at room temperature (22 °C), in the refrigerator (5 °C), and in the freezer (−20 °C), as analyzed by both methods, indicating the suitability of using this solvent for future formulations of BCT.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to them being part of a PhD thesis; they will be made available once the thesis has been published.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Alexander, A.; Dwivedi, S.; Ajazuddin; Giri, T.K.; Saraf, S.; Saraf, S.; Tripathi, D.K. Approaches for Breaking the Barriers of Drug Permeation through Transdermal Drug Delivery. *J. Control. Release* **2012**, *164*, 26–40. [CrossRef] [PubMed]
2. Lam, J.K.W.; Cheung, C.C.K.; Chow, M.Y.T.; Harrop, E.; Lapwood, S.; Barclay, S.I.G.; Wong, I.C.K. Transmucosal Drug Administration as an Alternative Route in Palliative and End-of-Life Care during the COVID-19 Pandemic. *Adv. Drug. Deliv. Rev.* **2020**, *160*, 234–243. [CrossRef] [PubMed]
3. Drug Bank Baricitinib: Uses, Interactions, Mechanism of Action. Available online: https://go.drugbank.com/drugs/DB11817 (accessed on 9 August 2022).
4. National Center for Biotechnology Information. PubChem Database. PubChem Compound Summary for CID 44205240, Baricitinib. Available online: https://pubchem.ncbi.nlm.nih.gov/compound/Baricitinib (accessed on 12 January 2022).
5. Nezamololama, N.; Fieldhouse, K.; Metzger, K.; Gooderham, M. Emerging Systemic JAK Inhibitors in the Treatment of Atopic Dermatitis: A Review of Abrocitinib, Baricitinib, and Upadacitinib. *Drugs Context* **2020**, *9*, 1–7. [CrossRef] [PubMed]
6. Nakashima, C.; Yanagihara, S.; Otsuka, A. Innovation in the Treatment of Atopic Dermatitis: Emerging Topical and Oral Janus Kinase Inhibitors. *Allergol. Int.* **2022**, *71*, 40–46. [CrossRef] [PubMed]
7. *EMA Ficha Técnica Baricitinib*; Agencia Europea de Medicamentos: London, UK, 2014; pp. 1–33.
8. U.S. FDA. *Prescribing Information OLUMIANT (Baricitinib) Tablets*; U.S. FDA: Silver Spring, MD, USA, 2022; Volume 50, pp. 1–25.
9. Marconi, V.C.; Ramanan, A.V.; de Bono, S.; Kartman, C.E.; Krishnan, V.; Liao, R.; Piruzeli, M.L.B.; Goldman, J.D.; Alatorre-Alexander, J.; de Cassia Pellegrini, R.; et al. Efficacy and Safety of Baricitinib for the Treatment of Hospitalised Adults with COVID-19 (COV-BARRIER): A Randomised, Double-Blind, Parallel-Group, Placebo-Controlled Phase 3 Trial. *Lancet Respir. Med.* **2021**, *9*, 1407–1418. [CrossRef]
10. Shi, J.G.; Chen, X.; Lee, F.; Emm, T.; Scherle, P.A.; Lo, Y.; Punwani, N.; Williams, W.V.; Yeleswaram, S. The Pharmacokinetics, Pharmacodynamics, and Safety of Baricitinib, an Oral JAK 1/2 Inhibitor, in Healthy Volunteers. *J. Clin. Pharmacol.* **2014**, *54*, 1354–1361. [CrossRef]
11. Reich, K.; Kabashima, K.; Peris, K.; Silverberg, J.I.; Eichenfield, L.F.; Bieber, T.; Kaszuba, A.; Kolodsick, J.; Yang, F.E.; Gamalo, M.; et al. Efficacy and Safety of Baricitinib Combined with Topical Corticosteroids for Treatment of Moderate to Severe Atopic Dermatitis: A Randomized Clinical Trial. *JAMA Dermatol.* **2020**, *156*, 1333–1343. [CrossRef]
12. Alshetaili, A.S. Solubility and Solution Thermodynamics of Baricitinib in Six Different Pharmaceutically Used Solvents at Different Temperatures. *Z. Phys. Chem.* **2019**, *233*, 1129–1144. [CrossRef]
13. Markham, A. Baricitinib: First Global Approval. *Drugs* **2017**, *77*, 697–704. [CrossRef]
14. Zheng, X.Q.; Huang, J.F.; Lin, J.L.; Zhu, Y.X.; Wang, M.Q.; Guo, M.L.; Zan, X.J.; Wu, A.M. Controlled Release of Baricitinib from a Thermos-Responsive Hydrogel System Inhibits Inflammation by Suppressing JAK2/STAT3 Pathway in Acute Spinal Cord Injury. *Colloids Surf. B Biointerfaces* **2021**, *199*, 111532. [CrossRef]
15. Hiraganahalli Bhaskarmurthy, D.; Evan Prince, S. Effect of Baricitinib on TPA-Induced Psoriasis like Skin Inflammation. *Life Sci.* **2021**, *279*, 119655. [CrossRef]
16. Anwer, M.K.; Ali, E.A.; Iqbal, M.; Ahmed, M.M.; Aldawsari, M.F.; Al Saqr, A.; Ansari, M.N.; Aboudzadeh, M.A. Development of Sustained Release Baricitinib Loaded Lipid-Polymer Hybrid Nanoparticles with Improved Oral Bioavailability. *Molecules* **2022**, *27*, 168. [CrossRef]
17. Ansari, M.J.; Alshahrani, S.M. Nano-Encapsulation and Characterization of Baricitinib Using Poly-Lactic-Glycolic Acid Co-Polymer. *Saudi Pharm. J.* **2019**, *27*, 491–501. [CrossRef]
18. Ma, T.Y.; Hollander, D.; Krugliak, P.; Katz, K. PEG 400, a Hydrophilic Molecular Probe for Measuring Intestinal Permeability. *Gastroenterology* **1990**, *98*, 39–46. [CrossRef]
19. Thakur, S.; Bajwa, N.; Baldi, A. New analytical methods for estimation of arteether by uv and fluorescence spectrophotometry: Development and validation. *J. Drug Deliv. Ther.* **2018**, *8*, 151–158. [CrossRef]
20. Karimi, M.; Mashreghi, M.; Saremi, S.S.; Jaafari, M.R. Spectrofluorometric Method Development and Validation for the Determination of Curcumin in Nanoliposomes and Plasma. *J. Fluoresc.* **2020**, *30*, 1113–1119. [CrossRef]
21. Fraihat, S.M.; al Khatib, H.S. Development and Validation of Spectrophotometric and Spectrofluorimetric Methods for Determination of Cilnidipine. *Trop. J. Pharm. Res.* **2020**, *19*, 1503–1509. [CrossRef]
22. Gandhi, S.V.; Kapoor, B.G. Development and Validation of UV Spectroscopic Method for Estimation of Baricitinib. *J. Drug Deliv. Ther.* **2019**, *9*, 488–491. [CrossRef]
23. Derayea, S.M.; Omar, M.A.; Abdel-Lateef, M.A.K.; Hassan, A.I. Development and Validation of a New Spectrofluorimetric Method for the Determination of Some Beta-Blockers through Fluorescence Quenching of Eosin Y. Application to Content Uniformity Test. *Open Chem.* **2016**, *14*, 258–266. [CrossRef]
24. Hadgraft, J. Passive Enhancement Strategies in Topical and Transdermal Drug Delivery. *Int. J. Pharm.* **1999**, *184*, 1–6. [CrossRef]

25. National Center for Biotechnology Information. PubChem Database. Diethylene Glycol Monoethyl Ether | C6H14O3—PubChem. Available online: https://pubchem.ncbi.nlm.nih.gov/compound/Diethylene-glycol-monoethyl-ether (accessed on 11 August 2022).
26. Sullivan, D.W.; Gad, S.C.; Julien, M. A Review of the Nonclinical Safety of Transcutol®, a Highly Purified Form of Diethylene Glycol Monoethyl Ether (DEGEE) Used as a Pharmaceutical Excipient. *Food Chem. Toxicol.* **2014**, *72*, 40–50. [CrossRef] [PubMed]
27. Pérez-González, N.; De Febrer, N.B.; Calpena-Campmany, A.C.; Nardi-Ricart, A.; Rodríguez-Lagunas, M.J.; Morales-Molina, J.A.; Soriano-Ruiz, J.L.; Fernández-Campos, F.; Clares-Naveros, B. New Formulations Loading Caspofungin for Topical Therapy of Vulvovaginal Candidiasis. *Gels* **2021**, *7*, 259. [CrossRef] [PubMed]
28. Predic Atkinson, J.; Maibach, H.I.; Dragicevic, N. Targets in Dermal and Transdermal Delivery and Classification of Penetration Enhancement Methods. In *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Drug Manipulation Strategies and Vehicle Effects*; Nina, D., Maibach, H.I., Eds.; Springer: Berlin/Heidelberg, Germany, 2015; pp. 93–108, ISBN 978-3-662-45013-0.
29. Mallandrich, M.; Fernández-Campos, F.; Clares, B.; Halbaut, L.; Alonso, C.; Coderch, L.; Garduño-Ramírez, M.L.; Andrade, B.; del Pozo, A.; Lane, M.E.; et al. Developing Transdermal Applications of Ketorolac Tromethamine Entrapped in Stimuli Sensitive Block Copolymer Hydrogels. *Pharm. Res.* **2017**, *34*, 1728–1740. [CrossRef] [PubMed]
30. Moussaoui, S.E.; Fernández-Campos, F.; Alonso, C.; Limón, D.; Mallandrich, M. Topical Mucoadhesive Alginate-Based Hydrogel Loading Ketorolac for Pain Management after Pharmacotherapy, Ablation, or Surgical Removal in Condyloma Acuminata. *Gels* **2021**, *7*, 8. [CrossRef] [PubMed]
31. Araújo, J.; Garcia, M.L.; Mallandrich, M.; Souto, E.B.; Calpena, A.C. Release Profile and Transscleral Permeation of Triamcinolone Acetonide Loaded Nanostructured Lipid Carriers (TA-NLC): In Vitro and Ex Vivo Studies. *Nanomedicine* **2012**, *8*, 1034–1041. [CrossRef]
32. Mallandrich, M.; Calpena, A.C.; Clares, B.; Parra, A.; Garcia, M.L.; Soriano, J.L.; Fernandez-Campos, F. Nano-Engineering of Ketorolac Tromethamine Platforms for Ocular Treatment of Inflammatory Disorders. *Nanomedicine* **2021**, *16*, 401–414. [CrossRef]
33. Silva-Abreu, M.; Espinoza, L.C.; Rodríguez-Lagunas, M.J.; Fábrega, M.J.; Espina, M.; García, M.L.; Calpena, A.C. Human Skin Permeation Studies with PPARγ Agonist to Improve Its Permeability and Efficacy in Inflammatory Processes. *Int. J. Mol. Sci.* **2017**, *18*, 2548. [CrossRef]
34. Sanz, R.; Clares, B.; Mallandrich, M.; Suñer-Carbó, J.; Montes, M.J.; Calpena, A.C. Development of a Mucoadhesive Delivery System for Control Release of Doxepin with Application in Vaginal Pain Relief Associated with Gynecological Surgery. *Int. J. Pharm.* **2018**, *535*, 393–401. [CrossRef]
35. Amores, S.; Domenech, J.; Colom, H.; Calpena, A.C.; Clares, B.; Gimeno, Á.; Lauroba, J. An Improved Cryopreservation Method for Porcine Buccal Mucosa in Ex Vivo Drug Permeation Studies Using Franz Diffusion Cells. *Eur. J. Pharm. Sci.* **2014**, *60*, 49–54. [CrossRef]
36. Gimeno, A.; Calpena, A.C.; Sanz, R.; Mallandrich, M.; Peraire, C.; Clares, B. Transbuccal Delivery of Doxepin: Studies on Permeation and Histological Investigation. *Int. J. Pharm.* **2014**, *477*, 650–654. [CrossRef]
37. Parra, A.; Mallandrich, M.; Clares, B.; Egea, M.A.; Espina, M.; García, M.L.; Calpena, A.C. Design and Elaboration of Freeze-Dried PLGA Nanoparticles for the Transcorneal Permeation of Carprofen: Ocular Anti-Inflammatory Applications. *Colloids Surf. B Biointerfaces* **2015**, *136*, 935–943. [CrossRef]
38. Limón, D.; Jiménez-Newman, C.; Calpena, A.C.; González-Campo, A.; Amabilino, D.B.; Pérez-García, L. Microscale Coiling in Bis-Imidazolium Supramolecular Hydrogel Fibres Induced by Release of a Cationic Serine Protease Inhibitor (ESI). *Chem. Commun.* **2017**, *53*, S1–S20. [CrossRef]
39. Limón, D.; Jiménez-Newman, C.; Rodrigues, M.; González-Campo, A.; Amabilino, D.B.; Calpena, A.C.; Pérez-García, L. Cationic Supramolecular Hydrogels for Overcoming the Skin Barrier in Drug Delivery. *ChemistryOpen* **2017**, *6*, 585–598. [CrossRef]
40. Limón, D.; Talló Domínguez, K.; Garduño-Ramírez, M.L.; Andrade, B.; Calpena, A.C.; Pérez-García, L. Nanostructured Supramolecular Hydrogels: Towards the Topical Treatment of Psoriasis and Other Skin Diseases. *Colloids Surf. B Biointerfaces* **2019**, *181*, 657–670. [CrossRef]
41. Committee for Medicinal Products for Human Use (CHMP). *European Medicines Agency Guideline on Bioanalytical Method Validation*; CHMP: London, UK, 2011; pp. 1–23.
42. Asociación Española de Farmacéuticos de la Industria (Ed.) *Validación de Métodos Analíticos*; Asociación Española de Farmacéuticos de la Industria: Madrid, Spain, 2001; ISBN 8489602336.
43. Kim, J.Y.; Kim, S.; Papp, M.; Park, K.; Pinal, R. Hydrotropic Solubilization of Poorly Water-Soluble Drugs. *J. Pharm. Sci.* **2010**, *99*, 3953–3965. [CrossRef]
44. USP 38. *General Notices and Requirements. Description and Solubility*; USP: Rockville, MD, USA, 2015.
45. Savjani, K.T.; Gajjar, A.K.; Savjani, J.K. Drug Solubility: Importance and Enhancement Techniques. *ISRN Pharm.* **2012**, *2012*, 1–10. [CrossRef]
46. Gupta, S.; Kesarla, R.; Omri, A. Formulation Strategies to Improve the Bioavailability of Poorly Absorbed Drugs with Special Emphasis on Self-Emulsifying Systems. *ISRN Pharm.* **2013**, *2013*, 1–16. [CrossRef]
47. Miyako, Y.; Khalef, N.; Matsuzaki, K.; Pinal, R. Solubility Enhancement of Hydrophobic Compounds by Cosolvents: Role of Solute Hydrophobicity on the Solubilization Effect. *Int. J. Pharm.* **2010**, *393*, 48–54. [CrossRef]

48. Sareen, S.; Joseph, L.; Mathew, G. Improvement in Solubility of Poor Water-Soluble Drugs by Solid Dispersion. *Int. J. Pharm. Investig.* **2012**, *2*, 12. [CrossRef]
49. EMA. *Assessment Report Olumiant*; EMA: London, UK, 2016.
50. Australian Government. *Australian Public Assessment Report for Baricitinib*; Australian Government: Canberra, Australia, 2019.
51. Kumar Bolla, P.; Clark, B.A.; Juluri, A.; Cheruvu, H.S.; Renukuntla, J. Pharmaceutics Evaluation of Formulation Parameters on Permeation of Ibuprofen from Topical Formulations Using Strat-M® Membrane. *Pharmaceutics* **2020**, *12*, 151. [CrossRef]
52. Gad, S.C.; Cassidy, C.D.; Aubert, N.; Spainhour, B.; Robbe, H. Nonclinical Vehicle Use in Studies by Multiple Routes in Multiple Species. *Int. J. Toxicol.* **2006**, *25*, 499–521. [CrossRef] [PubMed]
53. Silva-Abreu, M.; Espinoza, L.C.; Halbaut, L.; Espina, M.; García, M.L.; Calpena, A.C. Comparative Study of Ex Vivo Transmucosal Permeation of Pioglitazone Nanoparticles for the Treatment of Alzheimer's Disease. *Polymers* **2018**, *10*, 316. [CrossRef] [PubMed]
54. Gómez-Segura, L.; Parra, A.; Calpena, A.C.; Gimeno, Á.; Boix-Montañes, A. Carprofen Permeation Test through Porcine Ex Vivo Mucous Membranes and Ophthalmic Tissues for Tolerability Assessments: Validation and Histological Study. *Vet. Sci.* **2020**, *7*, 152. [CrossRef] [PubMed]
55. Miralles-Cardiel, E.; Silva-Abreu, M.; Calpena, A.C.; Casals, I. Development and Validation of an Hplc–Ms/Ms Method for Pioglitazone from Nanocarriers Quantitation in Ex Vivo and in Vivo Ocular Tissues. *Pharmaceutics* **2021**, *13*, 650. [CrossRef] [PubMed]
56. Gartner, L.P. Integument. In *Textbook of Histology*; Gartner, L.P., Ed.; Elsevier: Amsterdam, The Netherlands, 2021; pp. 333–354.
57. Kuo, S.-H.; Shen, C.-J.; Shen, C.-F.; Cheng, C.-M. Role of PH Value in Clinically Relevant Diagnosis. *Diagnostics* **2020**, *10*, 107. [CrossRef] [PubMed]
58. El-Hammadi, M.M.; Arias, J.L. Nanomedicine for Vaginal Drug Delivery. *Theory Appl. Nonparenter. Nanomed.* **2021**, 235–257. [CrossRef]
59. Rohan, L.C.; Sassi, A.B. Vaginal Drug Delivery Systems for HIV Prevention. *Am. Assoc. Pharm. Sci.* **2009**, *11*, 78–87. [CrossRef]
60. Gökmen, O.; Yeşilırmak, N.; Akman, A.; Güngör, S.G.; Yücel, A.E.; Yeşil, H.; Yıldız, F.; Sise, S.; Diakonis, V. Corneal, Scleral, Choroidal, and Foveal Thickness in Patients with Rheumatoid Arthritis. *Turk. J. Ophthalmol.* **2017**, *47*, 315–319. [CrossRef]
61. Aungst, B.; Svensson, C.K.; Gaudana, R.; Ananthula, H.K.; Parenky, A.; Mitra, A.K. Ocular Drug Delivery. *Am. Assoc. Pharm. Sci.* **2010**, *12*, 348–359. [CrossRef]
62. Leal, J.; Smyth, H.D.C.; Ghosh, D. Physicochemical Properties of Mucus and Their Impact on Transmucosal Drug Delivery. *Int. J. Pharm.* **2017**, *532*, 555–572. [CrossRef]
63. Boegh, M.; Nielsen, H.M. Mucus as a Barrier to Drug Delivery-Understanding and Mimicking the Barrier Properties. *Basic Clin. Pharmacol. Toxicol.* **2014**, *116*, 179–186. [CrossRef]
64. Choi, S.A.; Park, E.J.; Lee, J.H.; Min, K.A.; Kim, S.T.; Jang, D.-J.; Maeng, H.-J.; Jin, S.G.; Cho, K.H. Preparation and Characterization of Pazopanib Hydrochloride-Loaded Four-Component Self-Nanoemulsifying Drug Delivery Systems Preconcentrate for Enhanced Solubility and Dissolution. *Pharmaceutics* **2022**, *14*, 1875. [CrossRef]
65. Date, A.A.; Nagarsenker, M.S. Single-Step and Low-Energy Method to Prepare Solid Lipid Nanoparticles and Nanostructured Lipid Carriers Using Biocompatible Solvents. *Eur. J. Pharm. Res.* **2019**, *1*, 12–19. [CrossRef]
66. Lee, S.G.; Kang, J.B.; Kim, S.R.; Kim, C.J.; Yeom, D.W.; Yoon, H.Y.; Kwak, S.S.; Choi, Y.W. Enhanced Topical Delivery of Tacrolimus by a Carbomer Hydrogel Formulation with Transcutol P. *Drug Dev. Ind. Pharm.* **2016**, *42*, 1636–1642. [CrossRef]

Article 2

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Article

Lipid-based formulation of baricitinib for the topical treatment of psoriasis

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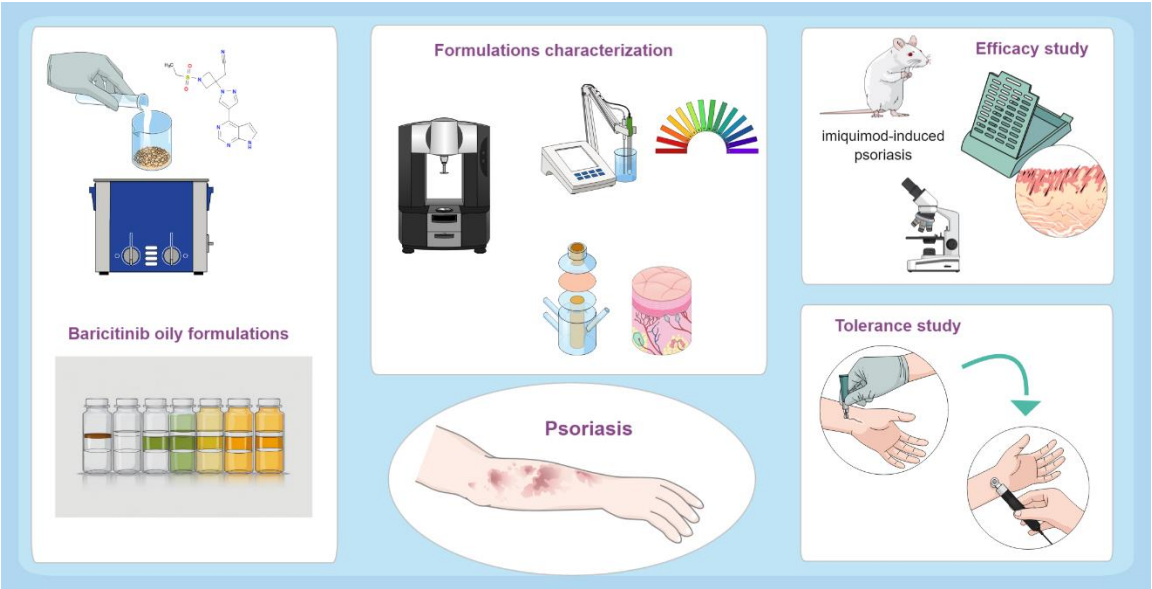

Summary

Psoriasis is a chronic skin condition characterized by red, itchy, and scaly patches, often caused by an overactive immune system. Psoriasis treatment often includes topical creams, light therapy, and systemic medications such as immunosuppressants. In this regard, baricitinib is commonly used to treat autoimmune diseases, including autoimmune skin conditions. However, oral administration can result in systemic adverse effects, making topical formulations a potentially more desirable option for localized treatment. This study aimed to develop a topical formulation of baricitinib based on lipid excipients to enhance localized delivery and minimize systemic side effects. The formulation was meticulously characterized through a series of physical, chemical, rheological, and biopharmaceutical assessments. Following this, its efficacy was evaluated using an imiquimod-induced psoriasis model in mice. Additionally, the tolerability of the formulation was thoroughly examined in vivo. The formulation exhibited satisfactory characteristics for skin delivery and maintained stability for at least one month. It demonstrated efficacy by significantly reducing the symptoms of psoriasis compared to the positive control. Additionally, the formulation was well-tolerated. In conclusion., this formulation shows promise as a treatment for psoriasis, with an apparently good safety profile.

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Lipid-Based Formulation of Baricitinib for the Topical Treatment of Psoriasis

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Abstract: Background: Baricitinib, commonly used for autoimmune diseases, is typically administered orally, which can lead to systemic adverse effects. A topical formulation could potentially offer localized therapeutic effects while minimizing these side effects. Objectives: This study focuses on developing a lipid-based topical formulation of baricitinib (BCT-OS) for treating psoriasis. Methods: The optimized formulation was then assessed for physical, chemical, and biopharmaceutical characterization. Furthermore, the anti-inflammatory efficacy of the formulation was tested in a model of psoriasis induced by imiquimod in mice, and its tolerance was determined by the evaluation of biomechanical skin properties and an inflammation test model induced by xylol in mice. Results: BCT-OS presented appropriate characteristics for skin administration in terms of pH, rheology, extensibility, and stability. The formulation also demonstrated a notable reduction in skin inflammation in the mouse model, and high tolerability without affecting the skin integrity. Conclusions: BCT-OS shows promise as an alternative treatment for psoriasis, offering localized therapeutic benefits with a potentially improved safety profile compared to systemic administration.

Keywords: baricitinib; lipid-based formulations; oily solutions; psoriasis; skin inflammation imiquimod

1. Introduction

Psoriasis is a chronic inflammatory skin disease with a worldwide prevalence of 1–3%, and is characterized by well-defined erythematous papules and plaques covered with silvery-white scales that can vary in intensity and be distributed in localized areas, including elbows, knees, lumbar region, armpits, groin, scalp, and nails [1]. This disease affects both men and women, but appears earlier in women and those with a family

history [2]. Due to the chronicity and severity of the disease, it can negatively impact the quality of life of patients at the mental, physical, and economic level [3]. The pathophysiology of psoriasis involves irregular keratinocyte proliferation and the infiltration of immune cells into both the dermis and epidermis. This process engages the innate and adaptive immune systems, dendritic cells and T cells playing important roles [4,5].

The treatments available for this disease include topical agents such as urea and salicylic acid ointments, corticosteroids alone or in combination with vitamin D analogues, retinoids, and calcineurin inhibitors. Other treatments used in psoriasis are phototherapy and oral or injectable treatments with steroids, retinoids, and biological drugs that target TNF, IL-12, IL-23, and IL-17 [6]. Several oral treatments are currently under development including phosphodiesterase (PDE) 4 inhibitors such as apremilast and roflumilast, oral interleukin (IL)-17 inhibitors such as tildrakizumab and Janus kinase (JAK) inhibitors such as tofacitinib, abrocitinib, peficitinib, and baricitinib (BCT) [7–11].

BCT is a drug that selectively inhibits JAK1/JAK2 tyrosine kinases. These kinases are located at the ends of cytokine receptors on the cell membrane and control the signal transduction of cytokines, including those in the IL-6, IL-10, IL-3, and IL-5 families. Each cytokine receptor is bound to two parallel isomers of JAK that exist as homodimers or heterodimers. Upon cytokine binding to their receptors, JAK undergoes phosphorylation, which in turn leads to the phosphorylation of STAT proteins (Signal transducer and transcription activator) in the cells. These STAT proteins are then transported to the nucleus where they interact directly with the cell's DNA to regulate gene expression [12]. This drug has been used in the management and treatment of rheumatoid arthritis, atopic dermatitis [13], and in recent years, in combination with other drugs for the treatment of COVID-19 [14]. Papp and coworkers evaluated the efficacy of oral baricitinib in managing psoriasis in a double-blind controlled study. The study demonstrated a significant improvement in the incidence and prevention of moderate to severe psoriasis in patients after 12 weeks of oral baricitinib treatment, resulting in a 75% reduction in the area [10].

BCT is commercially available in the form of tablets for oral administration (Olumiant®). The administration of this drug has adverse effects such as immunosuppression, the reactivation of herpes zoster, upper and lower respiratory tract infections, low red and white blood cell counts, significant cardiovascular adverse effects, and an increased risk of malignant neoplasms, and should be used with caution in patients over 65 years of age and diabetics. Before using baricitinib orally, a series of tests such as tuberculosis, HIV, viral hepatitis B or C, and liver function should be performed [15]. Figure 1 displays the chemical structure of BCT.

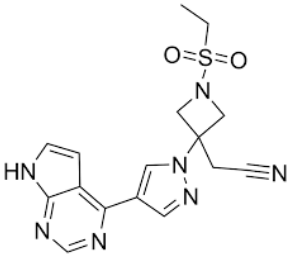

Figure 1. Chemical structure of baricitinib.

Administering drugs topically to the skin offers several advantages, including local treatment, minimized systemic side effects, and greater patient acceptance [16]. However, conventional topical formulations often face challenges such as poor solubility, limited skin penetration, and inadequate controlled release [17]. Drug delivery directly to the site of action through the skin can avoid first-pass metabolism and systemic degradation, thereby increasing drug bioavailability. There is growing interest in developing topical

treatments using appropriate vehicles to improve adherence in patients with chronic skin diseases such as psoriasis [18].

Lipid topical solutions offer notable advantages for the treatment of dermatological disorders in which the integrity of the skin is compromised since their components can act as moisturizers, humectants, and emollients, generating a protective barrier for the skin [19]. Topical oily solutions consist of a mixture of skin-friendly oily substances that help fill the spaces between intercorneocyte clusters to improve skin hydration, smoothness, softness, and suppleness. They frequently create an occlusive effect by preserving the skin's moisture through a hydrophobic barrier, which prevents transepidermal water loss. In addition, they are used as vehicles to solubilize water-insoluble drugs ensuring even application, protection, and delivery to deeper layers of the skin. [20].

Based on this background, the objective of this research was to design, develop, and characterize physically, chemically, and biopharmaceutically a lipid-based solution of BCT 5 mg/mL (BCT-OS) as an alternative topical treatment for psoriasis.

2. Materials and Methods

2.1. Chemicals and Reagents

The bulk powder of baricitinib (BCT) was sourced from Henrikang Biotech Co., Ltd. (Xi'an, China). Transcutol® P (diethylene glycol monoethyl ether), Labrafac® Lipophile WL 1349 (medium chain triglycerides), and Lauroglycol® 90 (propylene glycol monolaurate) were kindly donated by Gattefossé (Saint-Priest, France). Surfadone® LP 100 (N-Octyl-2-Pyrrolidone) was acquired from ISP (Yorkshire, UK), and imiquimod (IMQ) 50 mg/gr cream was obtained from Cantabria Labs (Madrid, Spain). Both distilled and purified water were produced using a Station 9000 purification unit.

2.2. Biological Material

Human skin was used to perform the ex vivo permeation studies. The human skin was sourced from abdominoplasty procedures in healthy women. The study protocol received approval from the Bioethics Committee of the Barcelona-SCIAS Hospital (Protocol N°002; dated 17 January 2020). The ex vivo skin was preserved at −20 °C until it was used in the permeation studies.

2.3. Analytical Method for Drug Quantification

BCT was determined by an HPLC method coupled to a fluorescence detector (HPLC-FLD). The system consisted of a Waters Alliance 2695 chromatograph (Waters, Milford, MA, USA) and a Jasco FP-1520 fluorescence detector (Jasco Corporation, Tokyo, Japan) at an excitation wavelength (EX) of 310 nm and an emission wavelength (EM) of 390 nm. The mobile phase was composed of 10 mM ammonium formate at pH 7: ACN (75:25 *v*/*v*). The flow rate was set at 1 mL/min under isocratic elution. The stationary phase was a Symmetry C18 column 4.6 × 75 mm, 3.5 µm (Waters Corporation, Cerdanyola del Vallès, Spain) and 10 µL of the samples was injected. A stock solution of 60 µg/mL BCT in Transcutol® P was prepared, then the calibration curve was obtained by sequential dilutions in Transcutol® P–PBS pH 7.4 (1:1, *v*/*v*) within the concentration range of 0.063–1 µg/mL.

2.4. Design and Preparation of Lipid-Based Topical Solutions of BCT 5 mg/mL

Different formulations were prepared using Transcutol® P as cosolvent to solubilize the drug. Additionally, different oily vehicles such as Labrafac® Lipophile WL 1349, Lauroglycol® 90, and Surfadone® LP 100 were used in different proportions (Table 1). First, Lauroglycol® and Surfadone® LP 100 were mixed in a ratio (5:2) using a magnetic stirrer for 10 min to ensure proper homogeneity. BCT was weighed in a vial and then the other components were added and sonicated in an ultrasound bath for 30 min to completely

solubilize the drug. Finally, the different formulations were equilibrated for 24 h at room temperature. The selection of the optimized formulation was based on the physical and chemical stability evaluated over 30 days of storage at 30 °C and 40 °C.

Table 1. Composition of the lipid-based formulations with different oily content. Quantities are expressed as percentages.

Formulations	Baricitinib (%)	Transcutol® P (%)	Labrafac®-Lipophile WL 1349 (%)	Lauroglycol® 90/Surfadone® LP 100 (5:2) (%)
T1	0.5	20	20	59.5
T2	0.5	20	40	39.5
T3	0.5	20	60	19.5
T4	0.5	40	20	39.5
T5	0.5	40	40	19.5
T6	0.5	60	20	19.5

2.5. Characterization of BCT-OS

2.5.1. pH

The pH of the lipid-based formulations was determined using a Crison micropH 2000 digital pH meter (Crison Instruments SA, Alella, Spain). To measure pH, 1 mL of formulation was combined with 30 mL of sterilized water and subjected to an ultrasound bath for 3 min. The electrode was directly immersed in the sample contained in a glass vial. Measurements were performed at room temperature 24 h after the formulations were prepared [21] and the results were reported as mean ± SD (n = 3).

2.5.2. Drug Content

The BCT content in the formulation was quantified using a spectrophotometer PerkinElmer UV/Vis (Shelton, CT, USA). BCT-OS was diluted in Transcutol® P to obtain the theoretical concentration within the standard solutions of the calibration curve (50–1.562 μg/mL). The calibration curve was prepared using Transcutol® P; a blank of the formulation was diluted following the same process of BCT-OS to use as a blank solution, and absorption measurements were performed using a quartz cuvette at 310 nm [22].

2.5.3. Rheological Properties

The rheological behaviour of the formulation was evaluated at 25 °C using a rotational rheometer Haake Rheostress® 1 (Thermo Fisher Scientific, Karlsruhe, Germany) equipped with a cone plate setup with a fixed lower plate and a mobile upper cone Haake C60/2° Ti (60 mm diameter, 2° angle). The rheometer was connected to a computer running Haake Rheowin® Data Manager v. 4.91 software (Thermo Electron Corporation, Karlsruhe, Germany) to carry out the test and Haake Rheowin® Data Manager v. 4.91 software (Thermo Electron Corporation, Karlsruhe, Germany) to analyze the obtained data. The shear rate ramp program (viscosity curves and flow curves) involved gradually increasing the shear from 0 to 100 s^{-1} over 3 min, maintaining a constant shear rate of 100 s^{-1}, for 1 min, and then gradually decreasing it back to 0 s^{-1} over 3 min. During this process, the shear stress (τ) was measured as a function of the shear rate (γ), providing the viscosity curves ($\eta = f(\gamma)$) and flow curves ($\tau = f(\gamma)$).

2.5.4. Extensibility

The extensibility assay was conducted to evaluate the spreading behavior of the topical product under varying degrees of pressure. This assay helps understanding the product's performance when different forces are applied. The extensibility was evaluated in triplicate using an extensometer (custom-designed and built in-house). The

extensiometer consists of a base with a mobile center that moves up and down with a lever. When the center is down, the base forms a hole where the formulation is allocated. A transparent and graded upper plaque is then placed on the base, and the lever is shifted to raise the center base compressing the formulation and causing it to spread. The surface area of the spreading is measured in centimeters. A volume of 200 μL of the formulation was placed in the center of the base of the extensometer. Subsequently, weights of 26, 28, 31, 36, and 46 g were sequentially applied to the formulation, and the extensibility was measured after 1 min at each weight. The extensibility was measured as the diameters of the spreading formulation [23]. The experimental data were analyzed and fitted to mathematical models by means of GraphPad Prism® version 6.0 (GraphPad Software Inc., San Diego, CA, USA).

2.6. In Vitro Release Study

In vitro release studies were carried out to assess the drug amount available from the selected formulation. To this end, Franz diffusion cells were used featuring a diffusion area of 0.64 cm^2 and a receptor chamber volume of 4.9 mL. A SpectraPor® regenerated cellulose dialysis membrane with a molecular cut-off weight of 14,000 Da (Sigma-Aldrich, Madrid, Spain) was hydrated for 24 h in methanol–water (1:1) to remove any residual impurities, then rinsed with Milli-Q water and afterwards soaked in receptor fluid for 4 h to equilibrate it before being mounted in the Franz diffusion cell (Crown Glass Company Inc., Jersey City, NJ, USA). Transcutol® P–PBS 7.4 (1:1, *v*/*v*) was used as the receptor medium that provided the sink conditions throughout the study based on the drug's solubility in the medium [22]. The assay was performed at 32 ± 0.5 °C by a circulating water jacket and the stirring speed was set at 500 r.p.m. to keep the contents uniform throughout the experiment. A total of 50 μL of the formulation was added to the donor compartment, and samples of 200 μL were withdrawn at the timepoints 3.0 h, 6.0 h, 8.30 h, 23.00 h, 26.30 h, 30.30 h, 40.00 h, 45.00 h, and 51.00 h. The same volume was promptly replaced by fresh Transcutol® P–PBS 7.4 (1:1, *v*/*v*) to keep the volume constant throughout the study. This experiment was carried out using 5 replicates for each formulation. The samples obtained were analyzed by HPLC-FLD (see Section 2.3).

2.7. Ex Vivo Permeation Study Using Human Skin

The ex vivo permeation studies are useful to evaluate the ability of the substances to penetrate the skin, providing insights into the diffusion characteristics of the drugs. The permeation study assay was conducted with ex vivo human skin, as the biological membrane, mounted on the Franz diffusion cells. The skin was clamped between the donor and receptor chambers. The receptor compartment was filled with Transcutol® P–PBS 7.4 (1:1, *v*/*v*) keeping sink conditions while being biocompatible with the skin [22]. The available diffusion area was 0.64 cm^2, and the receptor fluid was kept at 32 °C and stirred continuously at 500 r.p.m. Before applying the formulation, a sample of the receptor fluid was withdrawn as control and then, 50 μL of formulation was placed in the donor compartment and 200 μL of receptor fluid was collected at predetermined time intervals (1, 2, 3, 4, 5, 7.5, 18.5, 21.5, and 23.5 h) and replaced with an equivalent volume of fresh receptor fluid. The amount of drug in the samples obtained from this study was quantified according to the HPLC-FLD (see Section 2.3). The experiment included five replicates and the results were reported as mean ± SD.

The permeation profile was evaluated as the cumulative amount of BCT permeated (μg) versus time (h). Different biopharmaceutical parameters were estimated including the flux (J_{ss}, μg/(h/cm^2)), which was determined as the slope of the linear section of the permeation profile using linear regression analysis. Other parameters such as lag time (TL, h), permeability coefficient (K_P, (cm/h)), partition coefficient between vehicle and tissue (P_1, cm), and the diffusion coefficient (P_2, h^{-1}) were also estimated [24]. The theoretical concentration that would be achieved in plasma after the topical application of

the formulation in human (C_{ss}) was also predicted starting from the flux and taking into account BCT's plasma clearance [25] and a theoretical surface application of 10 cm^2.

After completing the permeation study, the amount of drug that remained in the skin was extracted as follows: the skin was removed from the Franz diffusion cells and washed with distilled water. The permeation area was punched and weighed. Then, the skin discs were immersed in 1 mL of Transcutol® P which acted as the extraction solvent. The drug was extracted from the skin by means of a sonication procedure for 10 min using an ultrasonic bath. The supernatant of samples was filtered and quantified by HPLC-FLD (see Section 2.3) to determine the amount of retained BCT in the tissue (Q_{ret}).

2.8. Efficacy Study: Imiquimod-Induced Psoriasis Model

2.8.1. Animals and Study Protocol

The study protocol received the approval from the Ethics Committee of BIOTERIO-UAEM de la Universidad Autónoma del Estado de Morelos (ref. 2023003 and date of approval on 12 May 2023). An imiquimod (IMQ)-induced psoriasis model was carried out using BALB/c mice to simulate the psoriasis symptoms in vivo. The animals remained in polypropylene cages at ambient temperature (20–25 °C) in a humidity-controlled (55%) environment with a light/dark cycle of 12 h, and given food and water ad libitum.

After a period of adaptation, the animals were randomly selected and divided into three groups according to experimental groups: negative control, positive control, and treatment group (n = 5 for each group). The mice in the negative control group were untreated healthy animals (no formulation or IMQ was applied). The animals in the positive group and BCT-OS group had psoriasis induced with 5 mg/mL of IMQ topically applied to the back skin and right ear for 6 consecutive days once daily and then the positive group was treated with 50 μL of PBS whereas the BCT-OS group was treated with 50 μL of the formulation once daily for another 6 days. At the end of the study, animals were euthanized by cervical dislocation and biopsy samples were extracted for histological analysis.

2.8.2. Evaluation of Thickness and Biomechanical Skin Properties

During the study, the ear thickness of the mice in each group was measured using a Pocket Thickness Gage 7309 (Mitutoyo Corp.; Kawasaki, Japan). This parameter was measured at different timepoints: day 0 (baseline), 6 (maximum inflammation), and 13 (last day of the trial). Additionally, the thickness of dorsal skin was measured after euthanizing the animals.

Biomechanical properties such as transepidermal water loss (TEWL) and stratum corneum hydration (SCH) were determined using a Tewameter measurement DermaLab1 module (Cortex Technology, Aalborg, Denmark) and a Corneometer CM-825 (Courage & Khazaka Electronics GmbH, Cologne, Germany), respectively. These parameters were measured at different timepoints: day 0 (basal), 6 (maximum inflammation), 8, 11, and 13 of the experiment.

2.8.3. Histological Analysis

The samples of skin from the ear and back of the mice were fixed in a 4% formaldehyde solution for 24 h at 2 °C and then washed with PBS 3 times, replacing it with fresh medium at one-hour intervals. Samples were dehydrated in ethanol solutions and subsequently cleared in xylene. Finally, samples were embedded in paraffin twice, cut in 5 μm sections, stained with hematoxylin and eosin and observed to evaluate the histomorphology using a light microscope Olympus BX41 equipped with an Olympus XC50 camera (Olympus Co., Tokyo, Japan).

2.9. Evaluation of Biopharmaceutical Parameters in Healthy and Psoriatic Skin

An ex vivo permeation study was conducted using mouse skin, both psoriatic-induced skin and healthy skin. For this purpose, skin samples from the positive and negative control of the efficacy study were used for this assay. Ex vivo permeation studies were performed using Franz diffusion cells following the methodology described in Section 2.7. At the end of the experiment the amount of drug retained in the skin was determined and biopharmaceutical parameters such as TL, K_p, P_1, and P_2 were also calculated according to Section 2.7.

2.10. Tolerance Study

2.10.1. Tolerance by Evaluation of Biomechanical Skin Properties

The tolerance of the formulation was evaluated using Blank-OS (formulation with no drug). The biomechanical skin parameters such as transepidermal water loss (TEWL) and corneal hydration (SCH) were evaluated using the DermaLab1 module (Cortex Technology, Aalborg, Denmark) and a Corneomter CM-825 (Courage & Khazaka Electronics GmbH, Cologne, Germany), respectively. Ten healthy volunteers in the age range of 25–40 years participated in this study with prior informed consent and approval from the ethics committee of the University of Barcelona (IRB00003099; approved on 20 March 2018). Volunteers were asked to refrain from using cosmetics for 6 h prior to the test. They had acclimatization periods of approximately 30 min. Then, 0.5 mL/cm^2 of Blank-OS was applied to the forearm of each volunteer. TEWL and SCH were measured in basal state and after formulation application at 10, 15, 30, 60, 120, and 180 min.

2.10.2. Tolerance Study in Mouse

The in vivo tolerance of BCT-OS was assessed using BALB/c mice in accordance with the Ethics Committee of BIOTERIO-UAEM de la Universidad Autónoma del Estado de Morelos (code 2023004 approved on 12 May 2023). A negative control (non-treated mouse), positive control (mouse treated with xylol), and mouse treated with BCT-OS were used in this experiment. A volume of 50 μL of the selected formulation or xylol was topically administered to the back skin previously shaved. After 2 h of exposition, the animals were euthanized by cervical dislocation, and samples of skin were removed for histological analysis following the method described in Section 2.8.3.

3. Results

3.1. Design and Preparation of Lipid-Based Topical Solutions of BCT 5 mg/mL

The formulations T1, T2, and T3 showed drug precipitation after one day of preparation, and thus they were discarded from the study. The formulations T4, T5, and T6 showed a homogenous appearance for 30 days at both 30 and 40 °C. However, under these same study conditions, parameters such as pH and drug content were more stable for T4, indicating that the qualitative and quantitative composition of this formulation has better drug compatibility compared to T5 and T6 (Tables 2 and 3), and therefore, T4 was selected as the final formulation of BCT-OS.

Table 2. Physical and chemical characterization of lipid-based topical solutions of BCT 5 mg/mL after one day of preparation and reevaluation at thirty days of preparation stored at 30 ± 2 °C/65 ± 5% RH.

Formulations	One Day		Thirty Days	
	pH	Drug Content (%)	pH	Drug Content (%)
T4	5.60 ± 0.12	99.02 ± 0.10	5.47 ± 0.09	98.95 ± 0.12
T5	5.57 ± 0.10	98.97 ± 0.18	5.06 ± 0.13	98.01 ± 0.13
T6	5.45 ± 0.15	99.08 ± 0.09	4.73 ± 0.07	98.16 ± 0.15

Table 3. Physical and chemical characterization of lipid-based topical solutions of BCT 5 mg/mL after one day of preparation and reevaluation at thirty days of preparation stored at 40 ± 2 °C/75 ± 5% RH.

Formulations	One Day		Thirty Days	
	pH	Drug Content (%)	pH	Drug Content (%)
T4	5.60 ± 0.12	99.02 ± 0.10	5.38 ± 0.07	98.87 ± 0.10
T5	5.57 ± 0.10	98.97 ± 0.18	4.92 ± 0.12	97.92 ± 0.16
T6	5.45 ± 0.15	99.08 ± 0.09	4.35 ± 0.15	97.89 ± 0.12

The selected formulation of BCT-OS was prepared by dissolving BCT in 40% Transcutol® P, 20% of Labrafac® Lipophile WL 1349, 28.2% Lauroglycol® 90, and 11.3 of Surfadone® LP 100) using a sonication process for 30 min until the complete solubilization of the drug.

3.2. Characterization of the Formulations

3.2.1. Rheological Properties

The rheological behavior of the BCT-OS formulation is shown in Figure 2. BCT-OS exhibited a typical Newtonian profile, in which the relationship between the shear stress and the shear rate r was linear with an absence of thixotropy. The viscosity remained constant with a value of 9.38 ± 0.01 mPa·s at 100 s^{-1} at 25 ± 0.1 °C.

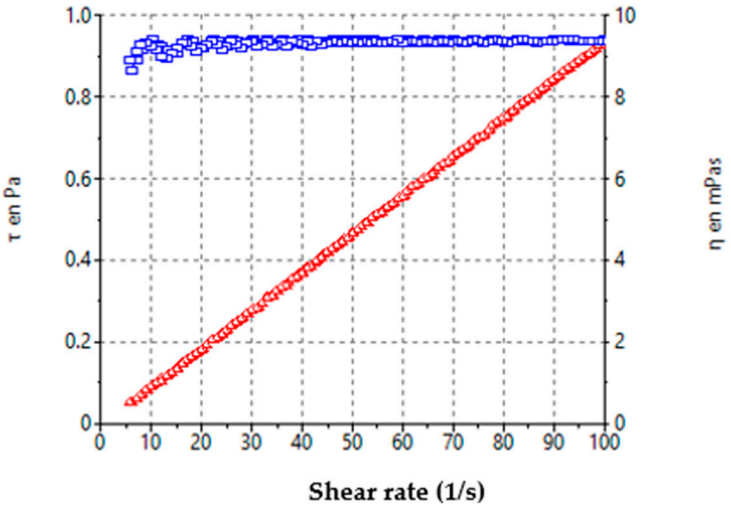

Figure 2. Rheological profile of BCT-OS. The viscosity curve is represented in blue color and the flow curve in red color.

3.2.2. Extensibility Assay

The extensibility assay involves subjecting the topical product to increasing weights to assess its ability to spread under pressure. This helps determine how the product behaves when applied with varying degrees of force, which can be important for user experience and product performance. The extensibility profile of BCT-OS followed a hyperbolic mathematical model with an r^2 value of 0.9445 (Figure 3). Its low viscosity favored the easy spreadability of the formulation as the different weights were added until reaching a maximum extensibility of about 20 cm^2.

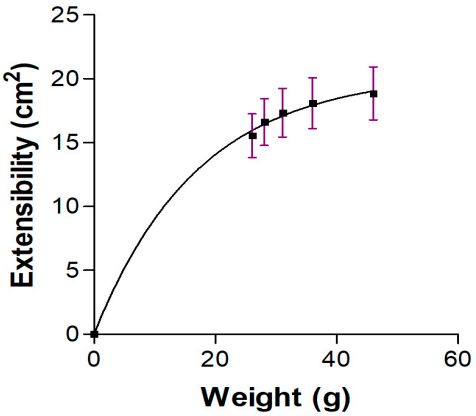

Figure 3. Extensibility profile of BCT-OS. Data are shown as the mean ± SD of three independent experiments (n = 3).

3.3. In Vitro Release Study

The drug release profile from the BCT-OS formulation is represented by the cumulative amounts of BCT released as a function of time, as shown in Figure 4. After 51 h of experiment, about 80% of the initial drug was released from the formulation.

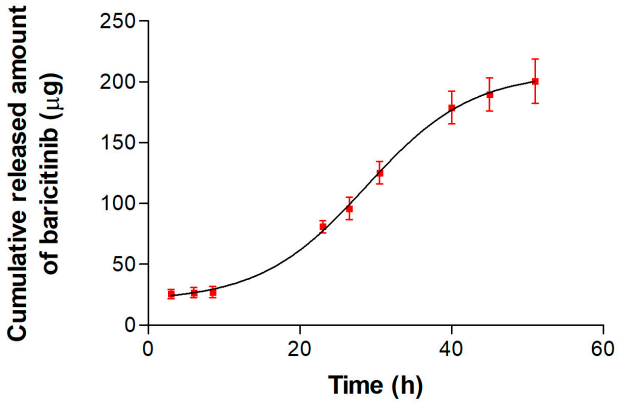

Figure 4. In vitro drug release profile of BCT from formulation. Data are presented as the mean ± SD (n = 5).

3.4. Ex Vivo Permeation Study Using Human Skin

Figure 5 shows the results of BCT permeation through human skin obtained from ex vivo studies. Only a minimal amount of drug was found in the samples extracted from the receptor fluid during the 23.5 h of study, indicating the difficulty of the drug in penetrating the deep layers of the skin.

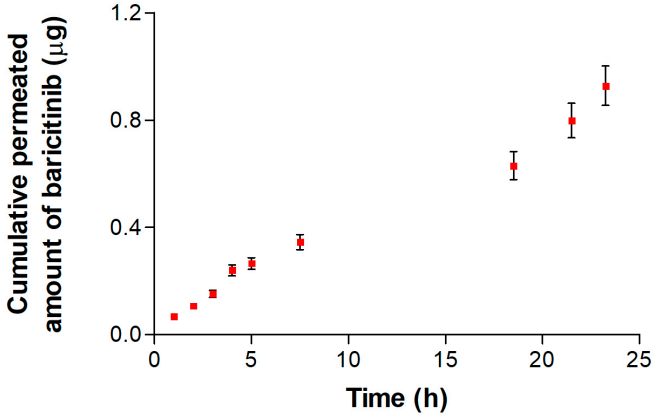

Figure 5. Permeation profile of BCT from the BCT-OS formulation through ex vivo human skin. Results are depicted as mean ± SD (n = 5).

Table 4 exhibits the permeation parameters calculated from the ex vivo permeation profile of BCT-OS. Results showed a drug flux (J_{ss}) of 0.10 ± 0.02 μg/(h/cm²), a permeability coefficient (K_p) of 0.19 ± 0.03 × 10^{-4} cm/h, vehicle/tissue partition coefficient (P_1) of 0.14 ± 0.02 × 10^{-4} cm, and a diffusion coefficient (P_2) of 1.40 ± 0.13 h^{-1}. Additionally, the time taken for BCT to appear in the receptor fluid at a steady rate (Tl) was 8.42 ± 0.78 h. The theoretical steady-state plasma concentration (C_{ss}) predicted was 0.06 ± 0.01 ng/mL, and the amount of BCT retained in the skin was 277.62 ± 52.75 μg/g skin/cm².

Table 4. Permeation parameters of BCT-OS through ex vivo human skin. Results are reported as mean ± SD (n = 5).

Parameters	Mean ± SD
J_{ss} (μg/(h/cm²))	0.10 ± 0.02
K_p (×10^{-4} cm/h)	0.19 ± 0.03
Tl (h)	8.42 ± 0.78
P_2 (h^{-1})	1.40 ± 0.13
P_1 (×10^{-4} cm)	0.14 ± 0.02
C_{ss} (ng/mL)	0.06 ± 0.01
Q_{ret} (μg/g skin/cm²)	277.62 ± 52.75

Abbreviations: J_{ss} (flux), K_p (permeability coefficient), Tl (lag time), P_1 (vehicle/tissue partition coefficient), P_2 (diffusion coefficient), C_{ss} (steady-state plasma concentration), and Q_{ret} (amount of drug retained in the tissue).

3.5. Efficacy Study: Imiquimod-Induced Psoriasis Model

The topical exposure of IMQ to the skin of mice for 6 days induced the development of scaly lesions, erythema, and edema, characteristic symptoms of psoriasis (Figure 6b,e). However, daily treatment for 6 days with the formulation decreased these symptoms and progressively improved the appearance of the skin (Figure 6c,f).

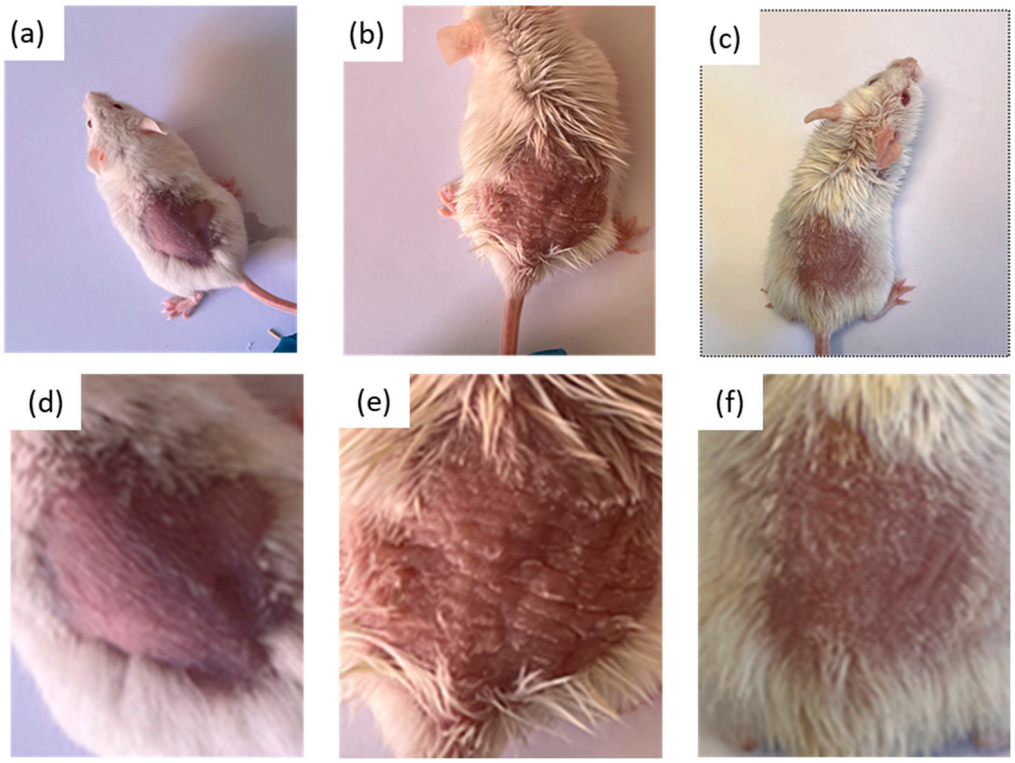

Figure 6. Photographs of the dorsal skin of mice on the last day of the experiment: negative control group (**a**,**d**); positive control group (**b**,**e**); and group treated with BCT-OS (**c**,**f**).

3.5.1. Evaluation of Thickness and Biomechanical Skin Properties

After inducing inflammation for 6 days with IMQ, a significant increase in the thickness of mice ears was observed compared to the baseline (day 0). However, topical treatment with BCT-OS for 6 days after inducing psoriasis significantly reduced this parameter, alleviating the skin edema (Figure 7A). The thickness evaluation of the dorsal skin excised after sacrificing the experimental animals also confirmed these results: the positive control exhibited a markedly higher thickness compared to the BCT-OS treated group, which showed a similar value to the negative control group (Figure 7B).

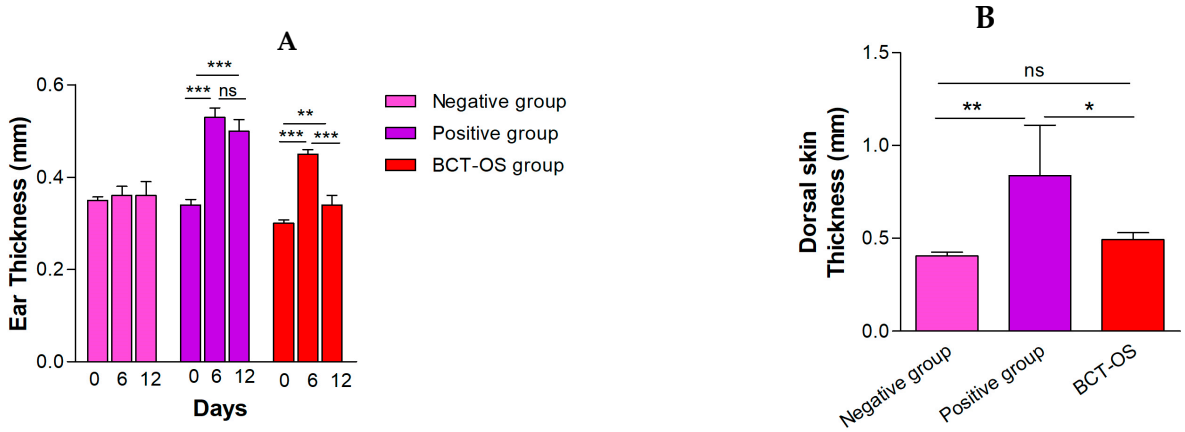

Figure 7. Evaluation of the skin thickness in (**A**) ear and (**B**) dorsal skin. Imiquimod was used to induce psoriasis in the positive group and in the BCT-OS group. Results are displayed as mean ± SD ($n = 5$). Significant statistical differences: * $p < 0.05$, ** $p < 0.01$, *** = $p < 0.001$, and ns, not significant.

Applying IMQ topically to the skin notably raised the TEWL value while decreasing the SCH value, indicating damage to the skin barrier's integrity, which aligns with the

dryness and scaly lesions observed macroscopically on the dorsal skin of the mice after the sixth day of the experiment (Figure 8). Despite this, the group treated topically with the formulation significantly reduced the TEWL value and increased the SCH levels, showing a restoration of the biomechanical properties of the skin (Figure 8C,F).

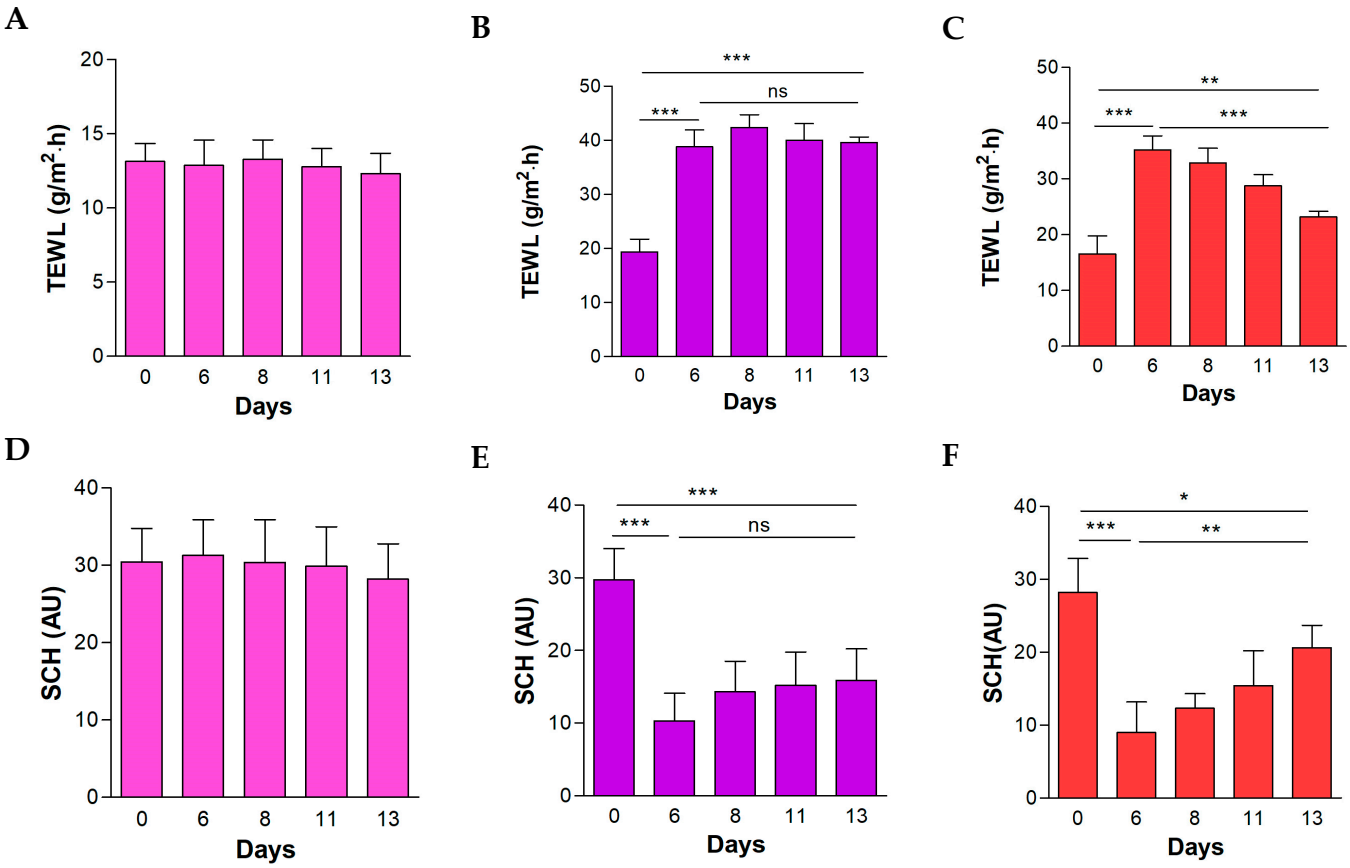

Figure 8. Evaluation of biomechanical skin properties in each experimental group using the imiquimod (IMQ)-induced psoriasis model. (**A**) Transepidermal water loss (TEWL) in the negative control group; (**B**) TEWL in the positive control group; (**C**) TEWL in the BCT-OS group; (**D**) stratum corneum hydration (SCH) in the negative control group; (**E**) SCH in the positive control group; and (**F**) SCH in the BCT-OS group. Results are expressed as mean ± SD (n = 5). Significant statistical differences: * $p < 0.05$, ** $p < 0.01$, *** = $p < 0.001$, and ns, not significant.

3.5.2. Histological Analysis

As shown in Figure 9A,D, healthy ear and dorsal skin in the negative control group exhibited an epidermis with normal appearance and an intact stratum corneum. The positive controls for ears and dorsal skin (Figure 9C,F) showed the presence of dilated blood vessels indicating an active inflammatory process induced by IMQ. The treatment with BCT-OS (Figure 9B,E) reduced the inflammation caused by IMQ showing a histological structure similar to the negative control with absent or very diminished dilated blood vessels.

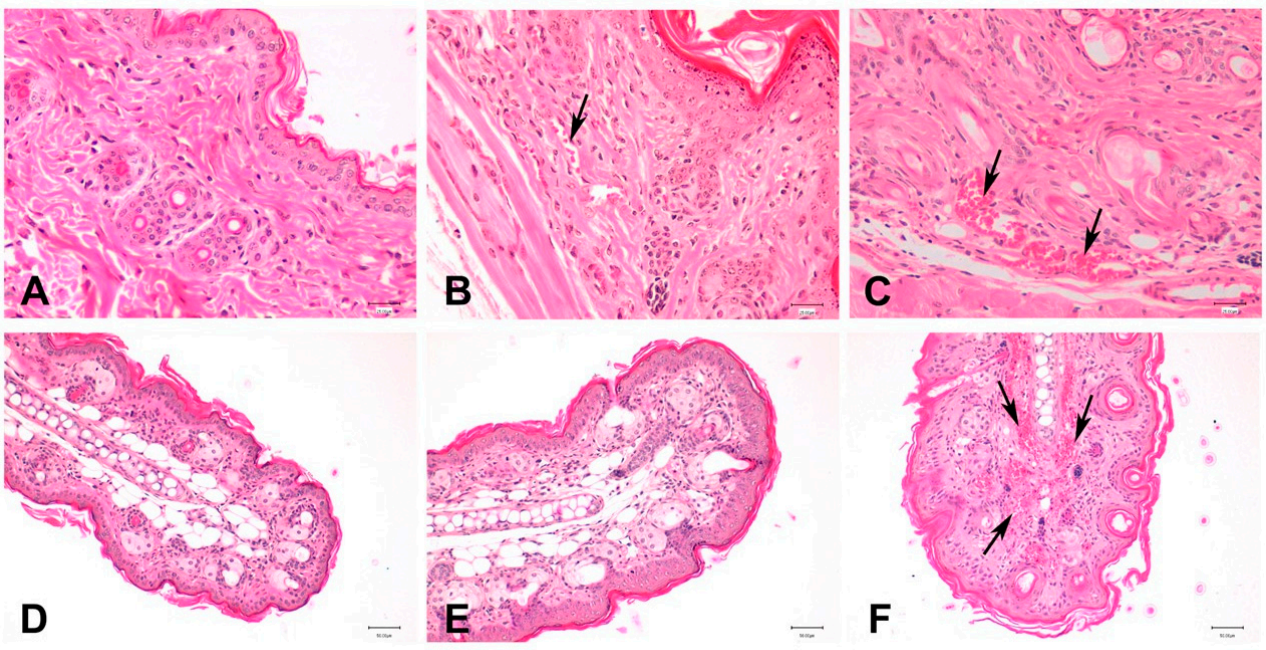

Figure 9. Histological images of ear and dorsal skin stained with hematoxylin and eosin. (**A**) Dorsal skin of the negative control group; (**B**) dorsal skin of the BCT-OS group; (**C**) dorsal skin of the positive control group; (**D**) ear skin of the negative control group; (**E**) ear skin of the BCT-OS group; and (**F**) ear skin of positive control group. Note the presence of dilated blood vessels (arrows) in the positive control, which are not observed or very diminished in the negative controls and treated tissues. Scale bar: (**A**–**C**) 25 μm; (**D**–**F**) 50 μm.

3.6. Evaluation of Biopharmaceutical Parameters in Healthy and Psoriatic Skin

Figure 10 depicts the amount of BCT permeated through psoriatic and healthy mouse skin after 25 h of assay. A higher permeation of BCT was observed through mouse skin affected by psoriasis (43.68 μg) than through healthy mouse skin (24.10 μg).

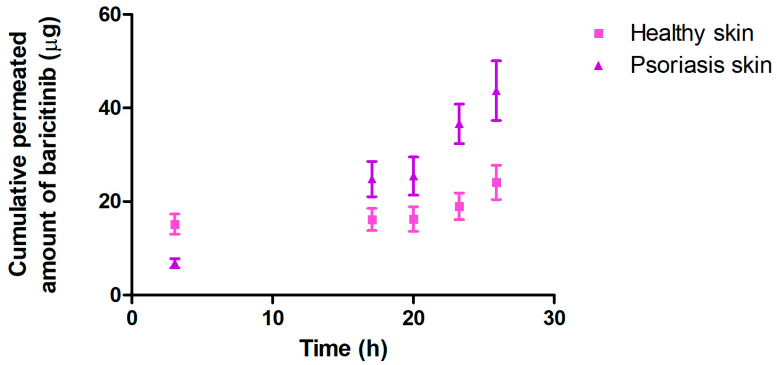

Figure 10. Permeation of BCT-OS through healthy and psoriatic ex vivo mouse skin. Results are expressed as mean ± SD (n = 5).

The biopharmaceutical analyses described in Table 5 revealed that the values of J_{ss} and Tl were considerably and statistically significantly higher for the ex vivo studies on psoriatic mouse skin compared to those performed on healthy skin. On the other hand, Q_{ret} showed no significant differences.

Table 5. Permeation parameters of BCT-OS through healthy and psoriasis mouse skin. Results are reported as mean ± SD (n = 5).

Parameters	Healthy Skin	Psoriasis Skin	Statistical Significance
J_{ss} (μg/h)	1.310 ± 0.138	3.100 ± 0.093	***
Tl (h)	7.89 ± 0.680	11.68 ± 1.250	***
Q_{ret} (μg/mg skin)	0.5320 ± 0.064	0.4880 ± 0.570	ns

Abbreviations: J_{ss} (flux), Tl (lag time), and Q_{ret} (amount of drug retained in the skin). *** = $p < 0.001$, and ns = not significant.

3.7. Tolerance Study

3.7.1. Tolerance Study by Evaluation of Biomechanical Skin Properties

Figure 11A illustrates a statistically significant decrease in the TEWL value after 10 min of application of the blank formulation to the skin. However, after 15 min, this parameter increased, progressively returning to the basal state. Regarding SCH, a significant increase was observed with respect to the basal state after 10, 15, 30, 60, and 120 min of topical treatment with the blank formulation. Nevertheless, a trend towards returning to the baseline state was noted after 3 h of the experiment (Figure 11B). The volunteers experienced no itching or pain during the evaluation of the formulation.

Figure 11. Assessment of the tolerance of the formulation in human individuals. (**A**) TEWL: transepidermal water loss and (**B**) SCH: stratum corneum hydration. Results are plotted as the mean and SD (n = 10). Statistically significant differences: * $p < 0.05$, ** $p < 0.01$, *** = $p < 0.001$, and ns, not significant.

3.7.2. Tolerance Study in Mouse

The tolerability of the formulation T4 was also evaluated in mice and compared to the negative control group, whose animals had been untreated, and to the positive control group, whose animals had been treated with xylol to cause inflammation.

Histologically, the negative control (Figure 12A) showed normal skin with a relatively thin epidermis and contiguous stratum corneum (SC). The topical application of xylol caused a loss of SC and epidermis. Finally, the skin treated topically with BCT-OS exhibited a similar pattern to that of the negative control group, with no signs of inflammation detected.

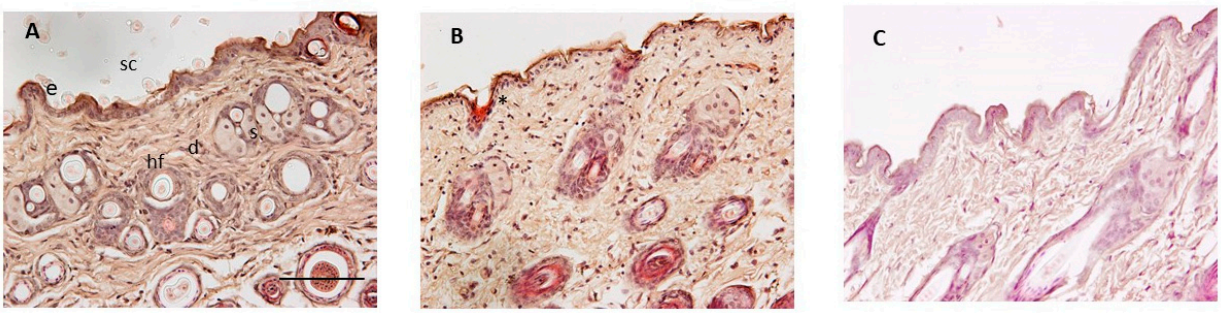

Figure 12. Representative histological sections of the mice skin in the tolerance study (original magnification, ×200). (**A**) Mouse skin of the negative control group; (**B**) group treated with xylol; and (**C**) group treated with BCT-OS. Skin structures: sc: stratum corneum e: viable epidermis; d: dermis; s: sebaceous gland; hf: hair follicle; and asterisk: loss of stratum corneum and epidermis. Scale bar = 100 μm.

4. Discussion

In this study, the incorporation of BCT into an oily solution was used as a strategy to locally treat the inflammation and characteristic symptoms of psoriasis. BCT-OS was formulated using pharmaceutical and cosmetic components suitable for dermal administration. The correct selection of excipients is important to provide substantial drug incorporation, along with the formulation's chemical and physical stability. Transcutol® P, an ethylene oxide derivative, was used as a solvent due to its high solubilizing capacity for the drug as well as due to its high biocompatibility with the skin [7]. This excipient has been extensively used in pharmaceutics and cosmetics for the preparation of creams, emulsions, gels, ointments, and topical solutions, encompassing a broad spectrum of uses, including analgesics, anti-inflammatories, antifungals, hormones, and veterinary products [26]. Labrafac® Lipophile WL 1349 and Lauroglycol® 90 were used as secondary oily vehicles since both have been previously used in the development of formulations for the treatment of psoriasis [19]. Finally, Surfadone® LP 100 was used as a penetration promotor to ensure the drug permeation through the stratum corneum [22].

BCT-OS exhibited appropriate organoleptic characteristics for topical application including excellent homogeneity and consistency, as well as a pH value of 5.60 which is biocompatible with the normal pH of the skin, guaranteeing non-irritating effects due to this parameter. Rheological properties of a topical formulation can modulate some biopharmaceutical parameters such as the release rate of the drug from its vehicle, as well as extensive coverage in the affected areas [27]. The rheological analysis confirmed the Newtonian behavior of BCT-OS at 25 °C (Figure 2), as anticipated in this type of formulation where there is a mixture of oils that create fairly fluid medications with low viscosity values. These low viscosity values facilitate packaging and administration by roll-on applications, or better yet, aerosolized to avoid touching the affected area [28]. The formulation showed high spreadability (Figure 3). The extensibility profile provides a measure of the deformation threshold of the vehicle. A topical formulation must exhibit high extensibility, since this means that it is not necessary to apply too much pressure to spread the product on the area to be treated [29–33] and considering that the product would be applied on inflamed psoriatic areas, it is ideal that its administration is easy and comfortable for the patient. The developed formulation is highly fluid and extensible, making it ideal for spray application. It can be easily applied to large areas of the body, such as the legs, abdomen, or arms, where psoriasis may occur.

The in vitro release study is relevant for evaluating the performance of topical products, to ensure that the drug is delivered effectively, since the drug release affects the permeation rate of the drug and therefore defines its bioavailability [34]. It is necessary to confirm that the drug is able to be released from the formulation before continuing with the skin permeation studies, since in the case that the drug does not permeate, we could

to be able to elucidate the reason, whether it is due to the inability of the drug to be released from the vehicle or the inability to permeate. In the event that the drug was not easily released, it would be necessary to make appropriate adjustments and reformulations. The results for BCT-OS showed that approximately 200 μg of BCT were released from the oily vehicle, which represents about 80% of the initial dose of the drug (Figure 4). This result indicates that this formulation is capable of releasing the drug, and therefore it will not hinder the drug's permeation through the skin. In other words, the drug release will not be a rate-limiting step in the drug's permeation through the skin.

The success of locally acting dermal treatments depends on the ability of the drug to penetrate the stratum corneum, diffuse through the deeper layers of the skin, and remain within the target area for the time necessary to exert its action [35,36]. Ex vivo permeation studies revealed that BCT persists in the skin and only a minimal amount of drug reached the receptor compartment (Figure 5), thereby demonstrating that BCT-OS could serve as a local cutaneous therapeutic approach without significant adverse systemic effects. Regarding the biopharmaceutical analysis (Table 4), the predominance of P_2 over P_1 facilitates the drug's diffusion through the epidermis and dermis. The high Tl value of 8.42 h means that during this time, the drug is diffusing through the skin and being distributed into it, ensuring a prolonged local effect and minimizing systemic adverse reactions. Previous studies have reported maximum plasma concentrations of 24.6 ng/mL after the oral administration of 2 mg BCT tablets [37]. Therefore, the low C_{ss} value of 0.06 ng/mL obtained in this study allows the avoidance of the systemic immunosuppressive effect of BCT, thus ensuring only a local anti-inflammatory activity. The high amount of BCT retained inside the skin (277.62 μg/g skin/cm^2) demonstrated that the drug is able to cross the stratum corneum and remain within the deeper layers of the skin, facilitating a local and prolonged effect of the drug. This result indicates that the oily solution enhances the retention of the drug in the target area, promoting its duration of action, which could consequently reduce the frequency of administration in patients.

In the IMQ-induced psoriasis model study, the results showed an increase in the thickness of the ear and dorsal skin of the animals as a result of the edema caused by the topical exposition to IMQ, but these thickness values decrease significantly with BCT-OS treatment. This effect could be explained by the anti-inflammatory activity already reported for BCT [38]. Biomechanical properties of the skin including TEWL and SCH were also evaluated. Specifically, in the negative control group (healthy mice), the TEWL values remained constant for the 13 days of the study. However, the topical application of IMQ on the skin caused an increase in these values of up to double compared to the basal state. Likewise, the SCH values decreased to approximately half of baseline values at the end of the experiment. This increase in TEWL and decrease in SCH is a consequence of the damage to the skin integrity caused by the topical exposure to IMQ, generating itching, redness, inflammation, and dryness, which was observed macroscopically on the affected skin (Figure 6) [39]. However, after applying the treatment with BCT-OS, the TEWL and SCH values were reestablished, which could be explained by the mixture of oils contained in the formulation, since oily substances help retain moisture and reduce the TEWL, improving skin hydration [40]. In addition, the application of oily formulations softens the surface of the skin by occupying the spaces between partially peeling skin scales (as in the case of psoriasis) and restores the capacity of intercellular lipid bilayers to absorb, retain, and redistribute water [41].

Regarding the histology in the positive control (Figure 9C,F), a dilation of the blood vessels can be observed, which is not observed in the ear treated with BST-OS (Figure 9E), and a decrease in these vessels can be seen on the back with the treatment applied (Figure 9E). These dilated blood vessels usually occur in inflammatory processes and have been reported in skin psoriasis, which manifests itself in the red color of the lesions as observed in this study [42–44]. Based on these encouraging findings, subsequent biochemical studies will be necessary and complementary to test the effectiveness of this formulation in animal models with the disease.

The ex vivo permeation study using healthy and psoriatic mouse skin showed that drug permeation and flux in psoriatic skin were about twice as high (43.68 μg and 3.100 μg/h, respectively) as those obtained in healthy skin (24.10 μg and 1.310 μg/h, respectively), expected results considering that the integrity of the skin is compromised in psoriasis, making it more susceptible to the transfer of substances across the skin barrier (Figure 10 and Table 5).

The tolerability of BCT-OS was studied by assessing the biomechanical properties of the skin, including TEWL and SCH, parameters widely used to evaluate the integrity of the skin barrier after being exposed to physical or chemical agents [45]. The results suggest that the vehicle is a safe formulation, as they showed a decrease in the TEWL values (more marked at 10 min) and an increase in SCH values after the application of the blank oily solution. These results could be due to the occlusive effect generated by the formulation which creates a hydrophobic barrier that blocks transepidermal water loss [20]. The mixture of oils could be covering small cracks in the skin providing a moisturizing effect, and a soothing and protective film. However, these TEWL and SCH values tended to revert to their baseline levels, concluding that there are no significant changes in terms of biomechanical parameters, and thus BCT-OS does not cause structural changes in the skin. This conclusion was supported by the tolerance study in mice, where histopathological analysis revealed no infiltration of inflammatory cells or notable changes in the skin compared to the negative control after the topical application of BCT-OS, which suggests that this formulation could be non-irritating and gentle on the skin.

5. Conclusions

Based on the findings of the research, the lipid-based formulation of baricitinib (BCT-OS) has demonstrated promising results in the topical treatment of psoriasis. This formulation showed controlled release and convenient skin permeation parameters, which are critical to ensure the drug reaches the desired target within the skin layers. Additionally, the BCT-OS formulation exhibited significant anti-inflammatory efficacy in an imiquimod-induced psoriasis model in mouse, suggesting its potential to alleviate symptoms of psoriasis. The formulation was well tolerated, with no adverse effects on skin integrity, highlighting its safety for prolonged use. These findings contribute significantly to the development of new alternatives to treatments for psoriasis. By offering localized therapeutic benefits and minimizing systemic side effects typically associated with oral administration, the BCT-OS formulation represents a potential breakthrough in psoriasis treatment, particularly for patients who experience adverse effects from systemic therapies.

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References

1. Armstrong, A.W.; Read, C. Pathophysiology, Clinical Presentation, and Treatment of Psoriasis: A Review. *JAMA* **2020**, *323*, 1945–1960. https://doi.org/10.1001/jama.2020.4006.
2. Virtanen, A.T.; Haikarainen, T.; Raivola, J.; Silvennoinen, O. Selective JAKinibs: Prospects in Inflammatory and Autoimmune Diseases. *BioDrugs* **2019**, *33*, 15–32. https://doi.org/10.1007/s40259-019-00333-w.
3. Kimball, A.B.; Jacobson, C.; Weiss, S.; Vreeland, M.G.; Wu, Y. The Psychosocial Burden of Psoriasis. *Am. J. Clin. Dermatol.* **2005**, *6*, 383–392. https://doi.org/10.2165/00128071-200506060-00005.
4. Greb, J.E.; Goldminz, A.M.; Elder, J.T.; Lebwohl, M.G.; Gladman, D.D.; Wu, J.J.; Mehta, N.N.; Finlay, A.Y.; Gottlieb, A.B. Psoriasis. *Nat. Rev. Dis. Primers* **2016**, *2*, 16082. https://doi.org/10.1038/nrdp.2016.82.
5. Słuczanowska-Głąbowska, S.; Ziegler-Krawczyk, A.; Szumilas, K.; Pawlik, A. Role of Janus Kinase Inhibitors in Therapy of Psoriasis. *J. Clin. Med.* **2021**, *10*, 4307. https://doi.org/10.3390/jcm10194307.
6. Griffiths, C.E.M.; Armstrong, A.W.; Gudjonsson, J.E.; Barker, J.N.W.N. Psoriasis. *Lancet* **2021**, *397*, 1301–1315. https://doi.org/10.1016/S0140-6736(20)32549-6.
7. Silva-Abreu, M.; Sosa, L.; Espinoza, L.C.; Fábrega, M.-J.; Rodríguez-Lagunas, M.J.; Mallandrich, M.; Calpena, A.C.; Garduño-Ramírez, M.L.; Rincón, M. Efficacy of Apremilast Gels in Mouse Model of Imiquimod-Induced Psoriasis Skin Inflammation. *Pharmaceutics* **2023**, *15*, 2403. https://doi.org/10.3390/pharmaceutics15102403.
8. Papp, K.A.; Menter, A.; Strober, B.; Langley, R.G.; Buonanno, M.; Wolk, R.; Gupta, P.; Krishnaswami, S.; Tan, H.; Harness, J.A. Efficacy and Safety of Tofacitinib, an Oral Janus Kinase Inhibitor, in the Treatment of Psoriasis: A Phase 2b Randomized Placebo-Controlled Dose-Ranging Study. *Br. J. Dermatol.* **2012**, *167*, 668–677. https://doi.org/10.1111/j.1365-2133.2012.11168.x.
9. Schmieder, G.J.; Draelos, Z.D.; Pariser, D.M.; Banfield, C.; Cox, L.; Hodge, M.; Kieras, E.; Parsons-Rich, D.; Menon, S.; Salganik, M.; et al. Efficacy and Safety of the Janus Kinase 1 Inhibitor PF-04965842 in Patients with Moderate-to-Severe Psoriasis: Phase II, Randomized, Double-Blind, Placebo-Controlled Study. *Br. J. Dermatol.* **2018**, *179*, 54–62. https://doi.org/10.1111/bjd.16004.
10. Papp, K.A.; Menter, M.A.; Raman, M.; Disch, D.; Schlichting, D.E.; Gaich, C.; Macias, W.; Zhang, X.; Janes, J.M. A Randomized Phase 2b Trial of Baricitinib, an Oral Janus Kinase (JAK) 1/JAK2 Inhibitor, in Patients with Moderate-to-severe Psoriasis. *Br. J. Dermatol.* **2016**, *174*, 1266–1276. https://doi.org/10.1111/bjd.14403.
11. Mansilla-Polo, M.; Gimeno, E.; Morgado-Carrasco, D. Roflumilast Tópico y Oral En Dermatología. Una Revisión Narrativa. *Actas Dermosifiliogr.* **2024**, *115*, 265–279. https://doi.org/10.1016/j.ad.2023.09.005.
12. O'Shea, J.J.; Schwartz, D.M.; Villarino, A.V.; Gadina, M.; McInnes, I.B.; Laurence, A. The JAK-STAT Pathway: Impact on Human Disease and Therapeutic Intervention. *Annu. Rev. Med.* **2015**, *66*, 311–328. https://doi.org/10.1146/annurev-med-051113-024537.
13. Reich, K.; Kabashima, K.; Peris, K.; Silverberg, J.I.; Eichenfield, L.F.; Bieber, T.; Kaszuba, A.; Kolodsick, J.; Yang, F.E.; Gamalo, M.; et al. Efficacy and Safety of Baricitinib Combined with Topical Corticosteroids for Treatment of Moderate to Severe Atopic Dermatitis: A Randomized Clinical Trial. *JAMA Dermatol.* **2020**, *156*, 1333–1343. https://doi.org/10.1001/jamadermatol.2020.3260.
14. Kalil, A.C.; Patterson, T.F.; Mehta, A.K.; Tomashek, K.M.; Wolfe, C.R.; Ghazaryan, V.; Marconi, V.C.; Ruiz-Palacios, G.M.; Hsieh, L.; Kline, S.; et al. Baricitinib plus Remdesivir for Hospitalized Adults with COVID-19. *N. Engl. J. Med.* **2021**, *384*, 795–807. https://doi.org/10.1056/NEJMoa2031994.
15. Eli Lilly and Company Olumiant (Baricitinib) Tablets 4 Mg, 2 Mg, 1 Mg. Available online: https://olumiant.lilly.com/ (accessed on 25 November 2023).
16. Cui, M.; Wiraja, C.; Chew, S.W.T.; Xu, C. Nanodelivery Systems for Topical Management of Skin Disorders. *Mol. Pharm.* **2021**, *18*, 491–505. https://doi.org/10.1021/acs.molpharmaceut.0c00154.
17. Kathe, K.; Kathpalia, H. Film Forming Systems for Topical and Transdermal Drug Delivery. *Asian J. Pharm. Sci.* **2017**, *12*, 487–497. https://doi.org/10.1016/j.ajps.2017.07.004.
18. Boehncke, W.-H.; Schön, M.P. Psoriasis. *Lancet* **2015**, *386*, 983–994. https://doi.org/10.1016/S0140-6736(14)61909-7.
19. Assalem, N.; Abd-allah, H.; Ragaie, M.H.; Ahmed, S.S.; Elmowafy, E. Therapeutic Potential of Limonene-Based Syringic Acid Nanoemulsion: Enhanced Ex-Vivo Cutaneous Deposition and Clinical Anti-Psoriatic Efficacy. *Int. J. Pharm.* **2024**, *660*, 124376. https://doi.org/10.1016/j.ijpharm.2024.124376.
20. Kurebayashi, A.K.; Phan, K.; Abdoh, A.; Andreo-Filho, N.; Lopes, P.S.; Mohammed, Y.; Leite-Silva, V.R. Strategic Approaches in Formulation Development for Atopic Dermatitis. *Cosmetics* **2024**, *11*, 113. https://doi.org/10.3390/cosmetics11040113.
21. Alía Fernández-Montes E. Fórmulas dermatológicas. Farmacia profesional : economía y gestión. 2003;17(2):70–5.

22. Mohammadi-Meyabadi, R.; Beirampour, N.; Garrós, N.; Alvarado, H.L.; Limón, D.; Silva-Abreu, M.; Calpena, A.C.; Mallandrich, M. Assessing the Solubility of Baricitinib and Drug Uptake in Different Tissues Using Absorption and Fluorescence Spectroscopies. *Pharmaceutics* **2022**, *14*, 2714. https://doi.org/10.3390/pharmaceutics14122714.
23. Folle, C.; Sánchez-López, E.; Mallandrich, M.; Díaz-Garrido, N.; Suñer-Carbó, J.; Halbaut, L.; Carvajal-Vidal, P.; Marqués, A.M.; Espina, M.; Badia, J.; et al. Semi-Solid Functionalized Nanostructured Lipid Carriers Loading Thymol for Skin Disorders. *Int. J. Pharm.* **2024**, *651*, 123732. https://doi.org/10.1016/j.ijpharm.2023.123732.
24. Abrego, G.; Alvarado, H.; Souto, E.B.; Guevara, B.; Bellowa, L.H.; Parra, A.; Calpena, A.; Garcia, M.L. Biopharmaceutical Profile of Pranoprofen-Loaded PLGA Nanoparticles Containing Hydrogels for Ocular Administration. *Eur. J. Pharm. Biopharm.* **2015**, *95*, 261–270. https://doi.org/10.1016/j.ejpb.2015.01.026.
25. Shi, J.G.; Chen, X.; Lee, F.; Emm, T.; Scherle, P.A.; Lo, Y.; Punwani, N.; Williams, W.V.; Yeleswaram, S. The Pharmacokinetics, Pharmacodynamics, and Safety of Baricitinib, an Oral JAK 1/2 Inhibitor, in Healthy Volunteers. *J. Clin. Pharmacol.* **2014**, *54*, 1354–1361. https://doi.org/10.1002/jcph.354.
26. Sullivan, D.W.; Gad, S.C.; Julien, M. A Review of the Nonclinical Safety of Transcutol®, a Highly Purified Form of Diethylene Glycol Monoethyl Ether (DEGEE) Used as a Pharmaceutical Excipient. *Food Chem. Toxicol.* **2014**, *72*, 40–50. https://doi.org/10.1016/J.FCT.2014.06.028.
27. Fernández-Campos, F.; Clares Naveros, B.; López Serrano, O.; Alonso Merino, C.; Calpena Campmany, A.C. Evaluation of Novel Nystatin Nanoemulsion for Skin Candidosis Infections. *Mycoses* **2013**, *56*, 70–81. https://doi.org/10.1111/j.1439-0507.2012.02202.x.
28. Sosa, L.; Calpena, A.; Silva-Abreu, M.; Espinoza, L.; Rincón, M.; Bozal, N.; Domenech, O.; Rodríguez-Lagunas, M.; Clares, B. Thermoreversible Gel-Loaded Amphotericin B for the Treatment of Dermal and Vaginal Candidiasis. *Pharmaceutics* **2019**, *11*, 312.
29. Ahmadi, N.; Rincón, M.; Silva-Abreu, M.; Sosa, L.; Pesantez-Narvaez, J.; Calpena, A.C.; Rodríguez-Lagunas, M.J.; Mallandrich, M. Semi-Solid Dosage Forms Containing Pranoprofen-Loaded NLC as Topical Therapy for Local Inflammation: In Vitro, Ex Vivo and In Vivo Evaluation. *Gels* **2023**, *9*, 448. https://doi.org/10.3390/gels9060448.
30. Latif, M.S.; Nawaz, A.; Asmari, M.; Uddin, J.; Ullah, H.; Ahmad, S. Formulation Development and In Vitro/In Vivo Characterization of Methotrexate-Loaded Nanoemulsion Gel Formulations for Enhanced Topical Delivery. *Gels* **2022**, *9*, 3. https://doi.org/10.3390/gels9010003.
31. Vlad, R.-A.; Dudici (Vlăgea), T.-C.; Syed, M.A.; Antonoaea, P.; Rédai, E.M.; Todoran, N.; Cotoi, C.-T.; Bîrsan, M.; Ciurba, A. Impact of the Preparation Method on the Formulation Properties of Allantoin Hydrogels: Evaluation Using Semi-Solid Control Diagram (SSCD) Principles. *Gels* **2024**, *10*, 58. https://doi.org/10.3390/gels10010058.
32. Khan, B.A.; Ahmad, N.; Alqahtani, A.; Baloch, R.; Rehman, A.U.; Khan, M.K. Formulation Development of Pharmaceutical Nanoemulgel for Transdermal Delivery of Feboxostat: Physical Characterization and in Vivo Evaluation. *Eur. J. Pharm. Sci.* **2024**, *195*, 106665. https://doi.org/10.1016/j.ejps.2023.106665.
33. Inoue, Y.; Suzuki, K.; Maeda, R.; Shimura, A.; Murata, I.; Kanamoto, I. Evaluation of Formulation Properties and Skin Penetration in the Same Additive-Containing Formulation. *Results Pharma Sci.* **2014**, *4*, 42–49. https://doi.org/10.1016/j.rinphs.2014.09.003.
34. Welin-Berger, K.; Neelissen, J.; Bergenståhl, B. In Vitro Permeation Profile of a Local Anaesthetic Compound from Topical Formulations with Different Rheological Behaviour—Verified by in Vivo Efficacy Data. *Eur. J. Pharm. Sci.* **2001**, *14*, 229–236. https://doi.org/10.1016/S0928-0987(01)00181-6.
35. Hagen, M.; Baker, M. Skin Penetration and Tissue Permeation after Topical Administration of Diclofenac. *Curr. Med. Res. Opin.* **2017**, *33*, 1623–1634. https://doi.org/10.1080/03007995.2017.1352497.
36. Marwah, H.; Garg, T.; Goyal, A.K.; Rath, G. Permeation Enhancer Strategies in Transdermal Drug Delivery. *Drug Deliv.* **2016**, *23*, 564–578. https://doi.org/10.3109/10717544.2014.935532.
37. Zhao, X.; Sheng, X.Y.; Payne, C.D.; Zhang, X.; Wang, F.; Cui, Y.M. Pharmacokinetics, Safety, and Tolerability of Single- and Multiple-Dose Once-Daily Baricitinib in Healthy Chinese Subjects: A Randomized Placebo-Controlled Study. *Clin. Pharmacol. Drug Dev.* **2020**, *9*, 952–960. https://doi.org/10.1002/cpdd.868.
38. Hiraganahalli Bhaskarmurthy, D.; Evan Prince, S. Effect of Baricitinib on TPA-Induced Psoriasis like Skin Inflammation. *Life Sci.* **2021**, *279*, 119655. https://doi.org/10.1016/j.lfs.2021.119655.
39. Balak, D.; Hajdarbegovic, E. Drug-Induced Psoriasis: Clinical Perspectives. *Psoriasis Targets Ther.* **2017**, *7*, 87–94. https://doi.org/10.2147/PTT.S126727.
40. Lodén, M. Effect of Moisturizers on Epidermal Barrier Function. *Clin. Dermatol.* **2012**, *30*, 286–296. https://doi.org/10.1016/j.clindermatol.2011.08.015.
41. Purnamawati, S.; Indrastuti, N.; Danarti, R.; Saefudin, T. The Role of Moisturizers in Addressing Various Kinds of Dermatitis: A Review. *Clin. Med. Res.* **2017**, *15*, 75–87. https://doi.org/10.3121/cmr.2017.1363.
42. Pober, J.S.; Sessa, W.C. Inflammation and the Blood Microvascular System. *Cold Spring Harb. Perspect. Biol.* **2015**, *7*, a016345. https://doi.org/10.1101/cshperspect.a016345.
43. Filipov, N.M. Chapter Six–Overview of Peripheral and Central Inflammatory Responses and Their Contribution to Neurotoxicity. In *Advances in Neurotoxicology*; Aschner, M., Costa, L.G., Eds.; Academic Press: Cambridge, MA, USA, 2019; Volume 3, pp. 169–193, ISBN 2468-7480.

44. Ubago-Rodríguez, A.; Quiñones-Vico, M.I.; Sánchez-Díaz, M.; Sanabria-de la Torre, R.; Sierra-Sánchez, Á.; Montero-Vílchez, T.; Fernández-González, A.; Arias-Santiago, S. Challenges in Psoriasis Research: A Systematic Review of Preclinical Models. *Dermatology* **2024**, *240*, 620–652. https://doi.org/10.1159/000538993.
45. Espinoza, L.C.; Silva-Abreu, M.; Calpena, A.C.; Rodríguez-Lagunas, M.J.; Fábrega, M.-J.; Garduño-Ramírez, M.L.; Clares, B. Nanoemulsion Strategy of Pioglitazone for the Treatment of Skin Inflammatory Diseases. *Nanomedicine* **2019**, *19*, 115–125. https://doi.org/10.1016/j.nano.2019.03.017.

DISCUSSION

Discussion

Preformulation of pharmaceutical products involves the investigation of a drug's physical and chemical properties before formulation development, intending to optimize drug delivery, stability, and efficacy (206). Solubility is one of the most critical properties evaluated during this since solubility data help select appropriate solvents, excipients, or carriers to enhance drug absorption (207). In the case of poorly soluble drugs like baricitinib, identifying solvents that improve solubility enables the development of more effective formulations since solubility directly impacts the bioavailability of drugs, particularly for oral and topical routes since the drug needs to dissolve in bodily fluids to be absorbed. Moreover, solubility studies are also critical for ensuring that the drug remains stable in the selected solvent over time, avoiding issues like precipitation or degradation, which are common challenges in formulation development (208).

In this doctoral thesis, the publication of the article entitled "Assessing the Solubility of Baricitinib and Drug Uptake in Different Tissues Using Absorption and Fluorescence Spectroscopies" was achieved. This work focused on addressing the challenge of baricitinib's poor water solubility. The study covered methods to improve the solubility of the drug, evaluate its uptake in different tissues, and develop analytical methods for drug quantification. For this purpose, the solubility of baricitinib was tested in various solvents, including oils, and permeation enhancers such as Transcutol, N-ethyl pyrrolidone, Capryol 90, Lauroglycol 90, Labrafac, Sebacic acid, Lauryl sulfate, limonene, 1-decanol, and isostearyl isostearate. The results highlighted

Transcutol and N-ethyl pyrrolidone as the most effective solvents for dissolving baricitinib. Transcutol is an ethylene oxide derivative which is a biocompatible solvent and permeation enhancer widely used in the development of pharmaceutical, and cosmetic products that in this study showed to be a viable solvent for enhancing the solubility and permeability of baricitinib in topical formulations (171). The significant improvement in the solubility of baricitinib when using Transcutol as a solvent could facilitate higher drug concentrations in the solution. This increased solubility is essential for enhancing the bioavailability of the drug, particularly in topical formulations (209).

Also, drug uptake and recovery studies were conducted to evaluate how much baricitinib penetrated various tissues and to assess the efficiency of drug extraction from these tissues. To that end, human skin and porcine tissues, including buccal, sublingual, vaginal, corneal, and scleral mucosae were incubated in a solution of baricitinib dissolved in Transcutol in controlled conditions of temperature, and later the concentration of baricitinib in the tissues was measured using absorption spectroscopy to determine how much of the drug had been absorbed. Finally, the drug was extracted from the tissues by sonication for 15 minutes and then quantified using either fluorescence spectroscopy or absorption spectroscopy, depending on the drug concentration. The recovery rate of the drug varied by tissue type. For instance, the buccal mucosa showed the highest uptake of baricitinib, while cornea and sublingual mucosae had the highest recovery rates. These studies were essential for determining both the penetration efficiency of the drug in different tissues and the effectiveness of the extraction method, enabling

accurate calculation of drug permeation rates. This could be due to the structure, and characteristics of each tissue (210).

In addition, the analytical methods including absorption spectroscopy and fluorescence spectroscopy for determining the concentration of baricitinib in different solvents were developed in this doctoral thesis. These methods were tailored to ensure they were precise, accurate, and capable of measuring baricitinib across a wide range of concentrations (211-212). The results showed that the absorption spectroscopy method was ideal for higher concentrations (6.25 µg/mL to 60 µg/mL), while fluorescence spectroscopy proved effective for detecting and quantifying much lower concentrations (0.078 µg/mL to 1.25 µg/mL), making both methods complementary in the study of drug solubility and tissue uptake. These validated methods offer several advantages including simplicity and low cost since they require relatively simple instrumentation, and they are a cost-effective method compared to more complex techniques like HPLC (high-performance liquid chromatography). This makes it accessible for routine analysis in pre-formulation studies. Moreover, these methods allow for quick detection and quantification of drug concentrations, making it efficient for large-scale experiments, and versatile for different stages of formulation (213).

With the results obtained in the first article published in this doctoral thesis, an oily formulation of baricitinib (BCT-OS) was developed and characterized resulting in a second article titled "Lipid-Based Formulation of Baricitinib for the Topical Treatment of Psoriasis". In this work, the design of a topical formulation of baricitinib was carried out using solvents like Transcutol P, Labrafac® Lipophile, Lauroglycol® 90, and Surfadone LP 100. These components were chosen for their solubilizing properties and

biocompatibility with the skin. Selecting the right excipients was crucial to ensure a high drug load and maintain the chemical and physical stability of formulations. Transcutol P was used as the solvent due to its strong solubilizing capacity, and skin Compatibility (171). Labrafac lipophile and lauroglycol 90 were secondary oily vehicles, chosen for their previous success in psoriasis formulations, while Surfadone LP100 was included as a permeation enhancer to promote drug absorption through the stratum corneum (214). The final formulation BCT-OS was selected based on stability, pH, and drug content after 30 days. This optimized formulation showed a homogeneous appearance, stability, and a pH of 5.60, compatible with the skin. Also, BCT-OS showed a Newtonian rheological behavior, suitable for easy application (215).

In vitro, release studies revealed that about 80% of the drug was released from the vehicle, suggesting that the BCT-OS is capable of releasing the drug and consequently it will not affect the drug permeation (216). The release kinetics followed a Boltzmann sigmoidal model, with an initial slow-release phase followed by controlled release (217). The ex vivo permeation studies allow researchers to investigate how well the drug penetrates through the stratum corneum and retains within the tissue, essential for achieving a localized therapeutic effect without significant systemic absorption (218-219). In the case of BCT-OS, the results demonstrated that only a minimal amount of baricitinib passed through the skin into the receptor chamber of Franz diffusion cells, while a significant portion remained within the skin. These results suggest that the formulation is well-suited for providing localized treatment for psoriasis, as it effectively retains the drug within the skin layers where the inflammation occurs minimizing the risk of systemic side

effects, which is a major advantage over oral administration of baricitinib, known for causing adverse effects. Moreover, the high amount of drug retained in the skin is likely due to the lipid-based formulation, which enhances the affinity of the drug for the stratum corneum and deeper layers of the skin. The occlusive properties of the oily components in the formulation create a barrier that helps retain the drug, ensuring that it stays in contact with the target tissues for an extended period. This prolonged retention contributes to a sustained release of the drug, allowing for more effective and long-lasting treatment of psoriasis (219).

Furthermore, the biopharmaceutical parameters, especially the amount of drug retained in the skin (Qret) and the theoretical plasma concentration (Css) provided additional insight into the performance of the formulation. The high Qret (277.62 µg/g skin/cm²) highlights the effectiveness of BCT-OS in ensuring the drug remains in the epidermis and dermis for a local, and prolonged effect of the drug. The low Css value of 0.06 ng/mL allows for avoiding the systemic immunosuppressive effect of BCT thus ensuring only a local anti-inflammatory activity (220). Therefore, the ex vivo permeation studies confirmed that BCT-OS is capable of crossing the stratum corneum and remaining in the deeper layers of the skin affected by psoriasis, offering a localized treatment option that minimizes systemic risks. This formulation provides prolonged drug retention, improving the overall therapeutic profile and reducing the frequency of application.

Regarding the efficacy of BCT-OS, its anti-inflammatory efficacy was tested using a psoriasis model induced by imiquimod (IMQ) in BALB/c mice. The study measured inflammation reduction and skin healing properties. The formulation showed therapeutic potential to reduce key symptoms of

psoriasis, including skin thickness, erythema, and edema, which are hallmark indicators of inflammation. These results align with baricitinib's known anti-inflammatory properties, as the drug inhibits Janus kinase (JAK) pathways involved in the inflammatory response associated with psoriasis (94,128). A notable outcome of the efficacy studies was the reduction in skin thickness in both the ear and dorsal regions of the mice suggesting the reduction of edema that is a characteristic symptom in psoriatic patients. By restoring the structure of the skin, BCT-OS demonstrates its potential to provide visible and symptomatic relief, making it a promising candidate for topical treatment. Additionally, the evaluation of biomechanical properties like trans epidermal water loss (TEWL) and stratum corneum hydration (SCH) further supported the efficacy of the formulation. IMQ application initially disrupted the skin barrier, leading to increased TEWL and reduced SCH values, mimicking the dehydration and dryness often seen in psoriasis (221). Treatment with BCT-OS reversed these effects, significantly restoring skin hydration and reducing water loss, suggesting an improvement in the overall barrier function of the skin. This ability to repair the skin is essential in psoriasis management, as the disease often leads to cracked, and irritated skin prone to infection (222). The histological analysis offered additional confirmation of the formulation’s effectiveness. In treated mice, there was a clear reduction in dilated blood vessels, a common sign of inflammation in psoriatic skin. This reduction in vascular dilation reflects the anti-inflammatory action of the BCT-OS formulation, reinforcing its potential as a localized treatment that targets inflammation at the source without causing systemic side effects (223). Overall, the efficacy studies highlight the strong therapeutic potential of BCT-OS in managing psoriasis symptoms. Its ability to reduce skin inflammation,

improve barrier function, and restore skin hydration points to a promising future for this topical formulation in the treatment of psoriasis.

The tolerance studies are critical in assessing the safety of new formulations for topical use, particularly in treating a chronic condition like psoriasis, where long-term use of medications can sometimes lead to skin irritation or other adverse effects. In this thesis, the biomechanical properties of the skin after topical application of BCT-OS were evaluated, including transepidermal water loss (TEWL) and stratum corneum hydration (SCH), both of which are key indicators of skin barrier integrity (224). After applying BCT-OS, the results showed a reduction in TEWL and an increase in SCH values, indicating that the formulation helps restore the skin barrier function by retaining moisture and minimizing water loss. This occlusive effect of the oily components in the formulation likely contributes to the creation of a protective layer over the skin, preventing dehydration, and improving overall skin hydration. This is particularly important in psoriasis, where the skin barrier is compromised, leading to symptoms such as dryness, itching, and redness. Additionally, the tolerability of the formulation was demonstrated by evaluation of structural changes in the skin using a mouse model. The histopathological analysis revealed no signs of inflammatory cell infiltration or skin irritation after the application of BCT-OS, indicating that the formulation is well-tolerated even with repeated use. Therefore, the results obtained in both tolerance experiments suggested that the formulation is safe for application over larger surface areas, as is often necessary for psoriasis treatment. The combination of non-irritating ingredients, and effective moisturization without compromising skin integrity makes BCT-OS a promising candidate for chronic use.

CONCLUSIONS

Conclusions

In this Thesis, a topical oily formulation containing baricitinib intended for treating psoriasis was developed and characterized.

- The solubility of baricitinib (BCT) in various excipients and media was thoroughly studied as part of the preliminary and pre-formulation phase.
- Transcutol enhanced BCT solubility and enabled its penetration into human skin, and porcine tissues (sclera, cornea, oral, sublingual, and vaginal)
- Analytical methods were developed and validated for quantifying BCT in Transcutol using absorption and fluorescence spectroscopies, allowing for drug detection across a wide concentration range.
- With a comprehensive understanding of the solubility, and permeability profile of baricitinib and the identification of the most suitable excipients, a lipid-based formulation was developed by investigating different proportions of the selected excipients.
- The developed formulation BCT-OS, exhibited satisfactory characteristics for skin delivery, including appropriate physical, chemical, and rheological attributes., and maintained stability for at least one month.
- Its efficacy was demonstrated using an imiquimod-induced psoriasis mice model, showing a significant reduction in psoriasis symptoms compared to the positive control.

- Differences in permeability between healthy and induced psoriasis skin were explored, revealing that drug permeation and flux in psoriatic mouse skin were approximately twice as high as in healthy skin., due to the compromised skin barrier in psoriasis.
- The formulation was well-tolerated as it showed a decrease in TEWL values, and increased SCH values, indicating no significant structural changes in the skin. The histopathological analysis in mice supported that BCT-OS is non-irritating and gentle on the skin.

REFERENCES

References

1. Parisi R., Iskandar I.Y., Kontopantelis E., Augustin M., Griffiths C.E., Ashcroft D.M. National., regional., and worldwide epidemiology of psoriasis: systematic analysis and modelling study. BMJ (2020). 369: m1590. https://doi.org/10.1136/bmj.m1590.

2. Michalek I., Loring B., John S. World Health Organization. Global report on psoriasis. World Health Organization., (2016). https://www.who.int/publications/i/item/9789241565189.

3. Burch P. R., Rowell N.R. Mode of inheritance in psoriasis. Arch Dermatol (1981). 117(5):251. https://doi.org/10.1001/archderm.1981.01650050005005.

4. Smith A.E., Kassab J.Y., Rowland Payne C.M., Beer W.E. Bimodality in age of onset of psoriasis., in both patients and their relatives. Dermatology (1993). 186:181-6. https://doi.org/10.1159/000247341

5. Ferrandiz C., Pujol R.M., Garcia-Patos V., Bordas X., Smandia J.A. Psoriasis of early and late onset: a clinical and epidemiologic study from Spain. J Am Acad Dermatol (2002). 46:867-73. https://doi.org/10.1067/mjd.2002.120470.

6. Henseler T., Christophers E. Psoriasis of early and late onset: characterization of two types of psoriasis vulgaris. J Am Acad Dermatol (1985). 13:450- 6. Stanford., CA: Stanford University Press., (1971). :25-34. https://doi.org/10.1016/S0190-9622(85)70188-0.

7. Global Psoriasis Atlas. Statistics. GPA. https://www.globalpsoriasisatlas.org/en/.

8. Global Burden of Disease Study 2010 (GBD 2010) Results by Cause 1990-2010. https://ghdx.healthdata.org/record/ihme-data/gbd-2010-results-cause-1990-2010.

9. Erratum: Global., regional., and national disability-adjusted life-years (DALYs) for 333 diseases and injuries and healthy life expectancy (HALE) for 195 countries and territories., 1990-2016: A systematic analysis for the Global Burden of Disease Study 2016. Lancet (2017). 390:1260–344. https://doi.org/10.1016/S0140-6736(17)32648-X.

10. Green A.C. Australian Aborigines and psoriasis. Australas J Dermatol (1984). 25(1):18-24. https://doi.org/10.1111/j.1440-0960.1984.tb00618.x.

11. Convit J. Investigation of the incidence of psoriasis amongst Latin-American Indians. In: Proceedings of 13th Congress on Dermatology. Amsterdam: Excerpta Medica., (1962). 196.

12. Fernández-Armenteros J. M., Gómez-Arbonés X., Buti-Solé M., Betriu-Bars A., Sanmartin-Novell V., Ortega-Bravo M., Martínez-Alonso M., Casanova-Seuma J. M.

Epidemiology of Psoriasis. A Population-Based Study. Actas Dermo-Sifiliográficas (2019). P 385-392. https://doi.org/10.1016/j.ad.2018.10.015.

13. Raharja A., Mahil S.K., Barker J.N. Psoriasis: a brief overview. Clinical Medicine., Journal of the Royal College of Physicians of London (2021). 21(3):170-173. https://doi.org/10.7861/CLINMED.2021-0257.

14. Smith C.H., Barker J.N. Psoriasis and its management. British Medical Journal (2006). 333. https://doi.org/10.1136/bmj.333.7564.380.

15. Sánchez Regaña M., Ojeda Cuchillero R., Umbert Millet I., Umbert Millet P., Lupo W., Solé Fernández J. El impacto psicosocial de la psoriasis. Actas Dermo-Sifiliograficas (2003). .,94(1-2):11-6. https://doi.org/10.1016/S0001-7310(03)79182-0.

16. Langley R. G. B., Krueger G. G., Griffiths C. E. M. Psoriasis: Epidemiology., clinical features., and quality of life. Annals of the rheumatic diseases (2005). 64(2)., 18-23. https://doi.org/10.1136/ard.2004.033217.

17. Vocalía Nacional de Dermofarmacia (Consejo General de Colegios de Farmacéuticos). Protocolos de Actuación Farmacéutica- PSORIASIS., https://www.farmaceuticos.com/wp-content/uploads/2022/06/Protocolo-Psoriasis.pdf.

18. Cunliffe T. The Primary Care Dermatology Society. LAST UPDATED: Dec 17., 2023. https://www.pcds.org.uk/clinical-guidance/psoriasis-an-overview.

19. Langley R.G., Krueger G.G., Griffiths C.E. Psoriasis: Epidemiology., clinical features., and quality of life., Annals of the Rheumatic Diseases (2005). 64(Suppl II): ii18-ii23. https://doi.org/10.1136/ard.2004.033217.

20. Martin B.A., Chalmers R.J., Telfer N.R. How great is the risk of further psoriasis following a single episode of acute guttate psoriasis? Arch Dermatol (1996). 132:717-18. https://doi.org/10.1001/archderm.1996.03890300147032.

21. Kim W.B., Jerome D., Frcpc M., Yeung J. Diagnosis and management of psoriasis. Canadian family physician., Can Fam Physician (2017). 63(4): 278-285. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5389757/pdf/0630278.pdf.

22. O'Doherty C.J., MacIntyre C. Palmoplantar pustulosis and smoking. British Medical Journal (Clinical research ed.) (1985). 28:861-4. https://doi.org/10.1136/bmj.291.6499.861.

23. Jyothi S.L., Krishna K.L., Ameena Shirin V.K., Sankar R., Pramod K., Gangadharappa H.V. Drug delivery systems for the treatment of psoriasis: Current status and prospects. Journal of Drug Delivery Science and Technology (2021). 62:102364. https://doi.org/10.1016/j.jddst.2021.102364.

24. Krueger G G., Ellis C. N. Psoriasis- Recent advances in understanding its pathogenesis and treatment. Journal of the American Academy of Dermatology (2005). 53: S94-100. https://doi.org/10.1016/j.jaad.2005.04.035.

25. Weinberg J.M., Lebwohl M. Advances in Psoriasis: A Multisystemic Guide 2nd ed. (2021). Book.

26. Dand N., Mahil S.K., Capon F., Smith C. H., Simpson M. A., Barker J. N. Acta Dermato-Venereologica (2020). 100(3)., 54-64. https://doi.org/10.2340/00015555-3384.

27. Schön M.P., Erpenbeck L. The interleukin-23/interleukin-17 axis links adaptive and innate immunity in psoriasis. Front Immunol (2018). 9:1323. https://doi.org/10.3389/fimmu.2018.01323.

28. Budu-Aggrey A., Brumpton B., Tyrrell J., Watkins S., Modalsli E. H., Celis-Morales C., Ferguson L. D., Vie G. Å., Palmer T., Fritsche L. G., Løset M., Nielsen J. B., Zhou W., Tsoi L. C., Wood A. R., Jones S. E., Beaumont R., Saunes M., Romundstad P. R., Siebert S., McInnes I. B., Elder J. T., Davey Smith G., Frayling T. M., Åsvold B. O., Brown S. J., Sattar N., Paternoster L. Evidence of a causal relationship between body mass index and psoriasis: A mendelian randomization study. PLoS Med (2019) .16: e1002739. https://doi.org/10.1371/journal.pmed.1002739.

29. Onoufriadis A., Simpson M.A., Pink A.E., Di Meglio P., Smith C. H., Pullabhatla V., Knight J., Spain S. L., Nestle F. O., Burden A. D., Capon F., Trembath R. C., Barker J. N. Mutations in IL36RN/ IL1F5 are associated with the severe episodic inflammatory skin disease known as generalized pustular psoriasis. American Journal of Human Genetics (2011). 89:432-7. https://doi.org/10.1016/j.ajhg.2011.07.022.

30. Trembath R.C., Clough R.L., Rosbotham J.L., Jones A.B., Camp R.D., Frodsham A., Browne J., Barber R., Terwilliger J., Lathrop G. M., Barker J. N. Identification of a major susceptibility locus on chromosome 6p and evidence for further disease loci revealed by a two stage genome-wide search in psoriasis. Human Molecular Genetics (1997). 6:813-20. https://doi.org/10.1093/hmg/6.5.813.

31. Nair R.P., Stuart P., Henseler T., Jenisch S., Chia N.V., Westphal E., Schork N. J., Kim J., Lim H. W., Christophers E., Voorhees J. J., Elder J. T. Localization of psoriasis-susceptibility locus PSORS1 to a 60-kb interval telomeric to HLA-C. American Journal of Human Genetics (2000). 66:1833-44., https://doi.org/10.1086/302932.

32. Veal C.D., Clough R.L., Barber R.C., Mason S., Tillman D., Ferry B., Jones A. B., Ameen M., Balendran N., Powis S. H., Burden A. D., Barker J.N., Trembath R. C. Identification of a novel psoriasis susceptibility locus at 1p and evidence of epistasis between PSORS1 and candidate loci. Journal of Medical Genetics (2001). 38:7-13. https://doi.org/10.1136/jmg.38.1.7.

33. Capon F., Novelli G., Semprini S., Clementi M., Nudo M., Vultaggio P., Mazzanti C., Gobello T., Botta A., Fabrizi G., Dallapiccola B. Searching for psoriasis susceptibility genes in Italy: genome scan and evidence for a new locus on chromosome 1. Journal of Investigative Dermatology (1999). 112:32-5. https://doi.org/10.1046/j.1523-1747.1999.00471.x.

34. Enlund F., Samuelsson L., Enerback C., Inerot A., Wahlstrom J., Yhr M., Torinsson Å., Riley J., Swanbeck G., Martinsson T. Psoriasis susceptibility locus in chromosome region 3q21 identified in patients from southwest Sweden. European Journal of Human Genetics (1999). 7:783-90. https://doi.org/10.1038/sj.ejhg.5200365.

35. Matthews D., Fry L., Powles A., Weber J., McCarthy M., Fisher E., Davies K. Williamson R. Evidence that a locus for familial psoriasis maps to chromosome 4q. Nature Genetics (1996). 14:231-3. https://doi.org/10.1038/ng1096-231.

36. Tomfohrde J., Silverman A., Barnes R., Fernandez-Vina M.A., Young M., Lory D., Morris L., Wuepper K. D., Stastny P., Menter A., Bowcock A. Gene for familial psoriasis susceptibility mapped to the distal end of human chromosome 17q. Science (1994). 264:1141-5. https://doi.org/10.1126/science.8178173.

37. Lee Y. A., Ruschendorf F., Windemuth C., Schmitt-Egenolf M., Stadelmann A., Nurnberg G., Ständer M. Wienker T. F.Reis A H. Genomewide scan in German families reveals evidence for a novel psoriasis-susceptibility locus on chromosome 19p13. American Journal of Human Genetics (2000). 67:1020-4. https://doi.org/10.1086/303075.

38. Paredes Paredes J.N., Novillo Flores M.D.C., Cárdenas Chávez A.B., Campuzano Rizzo B.L. Actualización terapéutica de la psoriasis. RECIMUNDO (2022). 6(2):318-29. https://doi.org/10.26820/recimundo/6.(2).abr.2022.318-329.

39. Cowden A., Van Voorhees A.S. Introduction: History of psoriasis and psoriasis therapy. In: Treatment of Psoriasis., Book., (2008). p. 1-9. https://doi.org/10.1007/978-3-7643-7724-3_1.

40. Olejnik M., Adamski Z., Dorocka-Bobkowska B. Psoriasis: From antiquity to the present. Indian Journal of Dermatology., Venereology and Leprology (2023). 89:149-153. https://doi.org/10.25259/IJDVL_1200_2021.

41. Gruber F., Kastelan M., Brajac I. Psoriasis treatment--yesterday., today., and tomorrow. Acta Dermatovenerologica Croatica (2004). 12(1):30-4.

42. Mahil S. K., Wilson N., Dand N., Reynolds N. J., Griffiths C. E., Emsley R., Marsden A., Evans I., Warren R. B., Stocken D., Barker J. N., Burden A. D., Smith C. H. Psoriasis treat to target: defining outcomes in psoriasis using data from a real-world., population based cohort study (the British Association of Dermatologists Biologics and Immunomodulators

Register., BADBIR). *British Journal of Dermatology* (2020). 182:1158-66. https://doi.org/10.1111/bjd.18333.

43. Warren R.B., Weatherhead S.C., Smith C.H., Exton L. S., Mohd Mustapa M. F., Kirby B., Yesudian P.D. British Association of Dermatologists guidelines for the safe and effective prescribing of methotrexate for skin disease 2016. British Journal of Dermatology (2016). 175:23- 44. https://doi.org/10.1111/bjd.14816.

44. Nast A., Smith C., Spuls P.I., Avila Valle G., Bata Csörgö Z., Boonen H., De Jong E., Garcia Doval I., Gisondi P., Kaur-Knudsen D., Mahil S., Mälkönen T., Maul J. T., Mburu S., Mrowietz U., Reich K., Remenyik E., Rønholt K. M., Sator P. G., Schmitt-Egenolf M., Sikora M., Strömer K., Sundnes O., Trigos D., Van Der Kraaij G., Yawalkar N., Dressler C. EuroGuiDerm Guideline on the systemic treatment of Psoriasis vulgaris – Part 2: specific clinical and comorbid situations. Journal of the European Academy of Dermatology and Venereology (2021). 35:281-317. https://doi.org/10.1111/jdv.16926.

45. Pathirana D., Ormerod A.D., Saiag P., Smith C.,Spuls P. I.,Nast A.,Barker J.,Bos J.D.,Burmester G. R.,Chimenti S.,Dubertret L.,Eberlein B.,Erdmann R.,Ferguson J.,Girolomoni G.,Gisondi P.,Giunta A.,Griffiths C.,Hönigsmann H.,Hussain M.,Jobling R.,Karvonen S. L.,Kemeny L.,Kopp I.,Leonardi C.,MacCarone M.,Menter A.,Mrowietz U.,Naldi L.,Nijsten T.,Ortonne J. P.,Orzechowski H. D.,Rantanen T.,Reich K.,Reytan N.,Richards H.,Thio H. B.,Van De Kerkhof P.,Rzany B. European S3-guidelines on the systemic treatment of psoriasis vulgaris.Journal of the European Academy of Dermatology and Venereology (2009). 23(Suppl 2):1-70.

46. National Institute for Health and Care Excellence. Ciclosporin. 2018. https://bnf.nice.org.uk/drug/ciclosporin.html. Accessed 2019.

47. Novartis Pharmaceuticals UK Ltd. Summary of product characteristics. Neoral solution. 2018. https://www.medicines.org.uk/emc/product/5300/smpc. Accessed 2019.

48. Nast A., Gisondi P., Ormerod A.D., Saiag P.,Smith C.,Spuls P.,I.Arenberger P.,Bachelez H.,Barker J.,Dauden E.,De Jong E. M.,Feist E.,Jacobs A.,Jobling R.,Kemény L.,Maccarone M.,Mrowietz U.,Papp K. A.,Paul C.,Reich K.,Rosumeck S.,Talme T.,Thio H. B.,Van De Kerkhof P.,Werner R. N.,Yawalkar N. European S3- guidelines on the systemic treatment of psoriasis vulgaris–update 2015–short version–EDF in cooperation with EADV and IPC. Journal of the European Academy of Dermatology and Venereology (2015). 29(12):2277-94. https://doi.org/10.1111/jdv.13354.

49. Gisondi P., Girolomoni G. Biologic therapies in psoriasis: a new therapeutic approach. Autoimmunity Reviews (2007). 6(8):515-9. https://doi.org/10.1016/j.autrev.2006.12.002.

50. Volc S., Ghoreschi K. Pathophysiological basis of systemic treatments in psoriasis. Journal der Deutschen Dermatologischen Gesellschaft (2016). 14(6):557-72. https://doi.org/10.1111/ddg.13050.

51. Afra T., Razmi T., Dogra S. Apremilast in psoriasis and beyond: Big hopes on a small molecule. Indian Dermatology Online Journal Indian (2019). 10(1): 1-12. https://doi.org/10.4103/idoj.IDOJ_437_18.

52. Bewley A., Shear N.H., Calzavara-Pinton P.G., Hansen J.B., Nyeland M.E., Signorovitch J. Calcipotriol plus betamethasone dipropionate aerosol foam versus apremilast., methotrexate., acitretin., or fumaric acid esters for the treatment of plaque psoriasis: A matching-adjusted indirect comparison. J Eur Acad Dermatol Venereol (2018). https://doi.org/10.1111/jdv.15369.

53. Crowley J., Thaçi D., Joly P., Peris K., Papp KA., Goncalves J., Day RM., Chen R., Shah K., Ferrándiz C., Cather JC. Long-term safety and tolerability of apremilast in patients with psoriasis: pooled safety analysis for C156 weeks from 2 phase 3., randomized., controlled trials (ESTEEM 1 and 2). J Am Acad Dermatol (2017). 77(2):310-17. e1. https://doi.org/10.1016/j.jaad.2017.01.052.

54. Reich K., Gooderham M., Green L., Bewley A., Zhang Z., Khanskaya I., Day R. M., Goncalves J., Shah K., Piguet V., Soung J. The efficacy and safety of apremilast., etanercept and placebo in patients with moderate-to-severe plaque psoriasis: 52-week results from a phase IIIb., randomized., placebo-controlled trial (LIBERATE). Journal of the European Academy of Dermatology and Venereology (2017). 31(3):507–17. https://doi.org/10.1111/jdv.14015.

55. Gooderham M., Papp K., Selective phosphodiesterase inhibitors for psoriasis: focus on apremilast. Bio- Drugs (2015). 29(5):327-39. https://doi.org/10.1007/s40259-015-0144-3.

56. Ling T. C., Clayton T. H., Crawley J., Exton L. S., Goulden V., Ibbotson S., McKenna K., Mohd Mustapa M. F., Rhodes L. E., Sarkany R., Dawe R.S. British Association of Dermatologists and British Photodermatology Group guidelines for the safe and effective use of psoralen-ultraviolet A therapy 2015. Br J Dermatol (2016). 174:24-55. https://doi.org/10.1111/bjd.14317.

57. Finlay A.Y., Ortonne J.P. Patient Satisfaction with Psoriasis Therapies: An Update and Introduction to Biologic Therapy. Journal of Cutaneous Medicine and Surgery (2004). 8(5):310-20. https://doi.org/10.1007/s10227-005-0030-6.

58. Nast A., Boehncke W.H., Mrowietz U., Ockenfels H. M., Philipp S., Reich K., Rosenbach T., Sammain A., Schlaeger M., Sebastian M., Sterry W., Streit V., Augustin M., Erdmann R.,

Klaus J., Koza J., Muller S., Orzechowski H. D., Rosumeck S., Schmid-Ott G., Weberschock T., Rzany B. S3-Guidelines on the treatment of psoriasis vulgaris (English version). Update. Journal of the German Society of Dermatology (2012). 10 (Suppl 2): S1 -95. https://doi.org/10.1111/j.1610-0387.2012.07919. x .

59. CatSalut. Adalimumab, etanercept, infliximab, ixekizumab, secukinumab and ustekinumab. https://catsalut.gencat.cat/ca/inici/.

60. Leonardi C.L., Kimball A.B., Papp K.A., Yeilding N., Guzzo C., Wang Y., Li S., Dooley L. T., Gordon K. B. Efficacy and safety of ustekinumab., a human interleukin-12/23 monoclonal antibody., in patients with psoriasis: 76-week results from a randomised., double-blind., placebo-controlled trial (PHOENIX 1). Lancet (2008). 371: 1665-74. https://doi.org/10.1016/S0140-6736(08)60725-4.

61. Menter A., Gottlieb A., Feldman S. R., Van Voorhees A., S.Leonardi C. L., Gordon K. B., Lebwohl M. Koo J. Y., Elmets C. A., Korman N. J., Beutner K. R., Bhushan R.. Guidelines of care for the management of psoriasis and psoriatic arthritis: Section 1. Overview of psoriasis and guidelines of care for the treatment of psoriasis with biologics. Journal of the American Academy of Dermatology (2008). 58:826 -50. https://doi.org/10.1016/j.jaad.2008.02.039.

62. Papp K. A., Langley R.G., Lebwohl M., Krueger G. G., Szapary P., Yeilding N., Guzzo C., Hsu M. C., Wang Y., Li S., Dooley L.T., Reich K. Efficacy and safety of ustekinumab., a human interleukin-12/23 monoclonal antibody., in patients with psoriasis: 52-week results from a randomised., double-blind., placebo-controlled trial (PHOENIX 2). The Lancet (2008). 371: 1675-84. https://doi.org/10.1016/S0140-6736(08)60726-6.

63. Callis Duffin K., Yeung H., Takeshita J., Krueger G. G., Robertson A. D., Troxel A. B., Shin D. B., Van Voorhees A. S., Gelfand J. M. Patient satisfaction with treatments for moderate-to-severe plaque psoriasis in clinical practice. British Journal of Dermatology (2014). 170: 672-80. https://doi.org/10.1111/bjd.12745.

64. Christophers E., Segaert S., Milligan G., Molta C. T., Boggs R. Clinical improvement and satisfaction with biologic therapy in patients with severe plaque psoriasis: results of a European cross-sectional observational study. Journal of Dermatological Treatment (2013). 24: 193-8. https://doi.org/10.3109/09546634.2012.697112.

65. Radtke M.A., Schafer I., Blome C., Augustin M. Patient benefit index (PBI) in the treatment of psoriasis-results of the National Care Study "PsoHealth". European Journal of Dermatology (2013). 23: 212-7. https://doi.org/10.1684/ejd.2013.1988.

66. Schaarschmidt M.L., Umar N., Schmieder A., Terris D. D., Goebeler M., Goerdt S., Peitsch W. K. Patient preferences for psoriasis treatments: impact of treatment

experience. Journal of the European Academy of Dermatology and Venereology (2013). 27: 187-98. https://doi.org/10.1111/j.1468-3083.2011.04440.x.

67. van Cranenburgh O.D., de Korte J., Sprangers M.A., De Rie M.A., Smets E. M. Satisfaction with treatment among patients with psoriasis: a web based survey study. British Journal of Dermatology (2013). 169: 398-405. https://doi.org/10.1111/bjd.12372.

68. Di Bonaventura M., Wagner S., Waters H., Carter C. Treatment patterns and perceptions of treatment attributes., satisfaction., and effectiveness among patients with psoriasis. Journal of Drugs in Dermatology (2010) .9: 938-44.

69. Blome C., Simianer S., Purwins S., Laass A., Rustenbach S. J., Schaefer I., Radtke M., Augustin M. Time needed for treatment is the major predictor of quality of life in psoriasis. Dermatology (2010). 221: 154-9. https://doi.org/10.1159/000313825.

70. Smith C.H., Yiu Z..Z..N., Bale T., Burden A. D., Coates L.C., Edwards W., MacMahon E., Mahil S. K., McGuire A., Murphy R., Nelson-Piercy C., Owen C. M., Parslew R., Uthman O. A., Woolf R. T., Manounah L., Ezejimofor M. C., Exton L.S., Mohd Mustapa M.F. British Association of Dermatologists guidelines for biologic therapy for psoriasis 2020: a rapid update. British Journal of Dermatology (2020). 183:628-37. https://doi.org/10.1111/bjd.19039.

71. Mahil S.K., Ezejimofor M.C., Exton L.S., Manounah L., Burden A.D., Coates L. C., de Brito M., McGuire A., Murphy R., Owen C.M., Parslew R., Woolf R.T., Yiu Z. Z., Uthman O.A., Mohd Mustapa M.F., Smith C.H. Comparing the efficacy and tolerability of biologic therapies in psoriasis: an updated network meta-analysis. British Journal of Dermatology (2020). 183:638-49. https://doi.org/10.1111/bjd.19325.

72. Dand N., Duckworth M., Baudry D., Russell A., Curtis C. J., Lee S. H., Evans I., Mason K. J., Alsharqi A., Becher G., Burden A. D., Goodwin R. G., McKenna K., Murphy R., Perera G. K., Rotarescu R., Wahie S., Wright A., Reynolds N. J., Warren R.B., Griffiths C. E., Smith C. H., Simpson M. A., Barker J. N., Benham M., Hussain S., Kirby B., Lawson L., McElhone K., Ormerod A., Owen C., Barnes M. R., Di Meglio P., Emsley R., Evans A., Payne K., Stocken D. HLA-C*06:02 genotype is a predictive biomarker of biologic treatment response in psoriasis. Journal of Allergy and Clinical Immunology (2019). 143:2120-30. https://doi.org/10.1016/j.jaci.2018.11.038.

73. Gniadecki R., Kragballe K., Dam T.N., Skov L. Comparison of drug survival rates for adalimumab., etanercept and infliximab in patients with psoriasis vulgaris. British Journal of Dermatology (2011). 164: 1091-6. https://doi.org/10.1111/j.1365-2133.2011.10213.x.

74. Levin A.A., Gottlieb A.B., Au S.C. A comparison of psoriasis drug failure rates and reasons for discontinuation in biologics vs conventional systemic therapies. Journal of Drugs in Dermatology (2014). 13: 848-53. PMID: 25007369.

75. Brunasso A.M., Puntoni M., Massone C. Drug survival rates of biologic treatments in patients with psoriasis vulgaris. British Journal of Dermatology (2012). 166: 447-9. https://doi.org/10.1111/j.1365-2133.2011.10557.x.

76. Bissonnette R., Stein Gold L., Rubenstein D.S., Tallman A.M., Armstrong A. Tapinarof in the treatment of psoriasis: a review of the unique mechanism of action of a novel therapeutic aryl hydrocarbon receptor–modulating agent. Journal of the American Academy of Dermatology (2021). 84(4):1059-1067. https://doi.org/10.1016/j.jaad.2020.10.085.

77. Griffiths C.E.M., Armstrong A.W., Gudjonsson J.E., Barker J.N.W.N. Psoriasis. The Lancet (2021). 397(10281):1301-1315. https://doi.org/10.1016/S0140-6736(20)32549-64.

78. Lebwohl M.G., Tan M.H., Meador S.L., Singer G. Limited application of fluticasone propionate ointment., 0.005% in patients with psoriasis of the face and intertriginous areas. Journal of the American Academy of Dermatology (2001). 44(1):77-82. https://doi.org/10.1067/mjd.2001.1100465.

79. Jeffrey M., Weinberg., Mark Lebwohl. Advances in Psoriasis., Textbook (2021). https://link.springer.com/book/10.1007/978-3-030-54859-9.

https://doi.org/10.1007/978-3-030-54859-9.

80. Katare O., Raza K., Singh B., Dogra S. Novel drug delivery systems in topical treatment of psoriasis: Rigors and vigors. Indian Journal of Dermatology., Venereology and Leprology (2010). 76(6):612-21. https://doi.org/10.4103/0378-6323.72451.

81. Morganti P., Ruocco E., Wolf R., Ruocco V. Percutaneous absorption and delivery systems. Clinics in Dermatology (2001). 19(4):489-501. https://doi.org/10.1016/S0738-081X(01)00183-3.

82. Vincent N., Ramya D.D., Vedha H.B. Progress in psoriasis therapy via novel drug delivery systems. Dermatology Reports (2014). 6(1):5451-5451. https://doi.org/10.4081/dr.2014.5451.

83. Callender S.P., Mathews J.A., Kobernyk K., Wettig S.D. Microemulsion utility in pharmaceuticals: Implications for multi-drug delivery., International Journal of Pharmaceutics (2017). P. 425-42. https://doi.org/10.1016/j.ijpharm.2017.05.005.

84. El-Zaafarany G.M., Nasr M. Insightful exploring of advanced nanocarriers for the topical/transdermal treatment of skin diseases. Pharmaceutical Development and Technology (2021). 26(10):1136-57. https://doi.org/10.1080/10837450.2021.2004606.

85. Al-Salama Z.T., Scott LJ. Baricitinib: A Review in rheumatoid arthritis. Adis Drug Evaluation (2018). 78:761-772. https://doi.org/10.1007/s40265-018-0908-4.

86. Mannurkar M.M., Hamrapurkar P.D. Development and validation of RP-HPLC method for Baricitinib using Quality by design approach and its application to stability studies. International Journal of Pharmaceutical Quality Assurance (2021). 12(1):40-47. https://doi.org/10.25258/ijpqa.12.1.6.

87. Jain S., Mateen S., M. Iqbal A.A., Singh R.M., Warde S.U., Munipalli V. K., Jadhav R. Analytical Method Development and Validation for Estimation of Baricitinib in Bulk and Formulation. Journal of Chemical Health Risks (2023). 13(1)., 212-220.

88. Mullard A. FDA approves Eli Lilly's baricitinib. Nat Rev Drug Discov (2018). 17(7):460. https://doi.org/10.1038/nrd.2018.112.

89. Richardson P.J., Corbellino M., Stebbing J. Baricitinib for COVID-19: suitable treatment? – Authors' reply. *The Lancet Infectious Diseases* (2020). https://doi.org/10.1016/S1473-3099(20)30270-X.

90. Rizk J.G., Forthal D.N., Kalantar-Zadeh K., Mehra M.R., Lavie C.J., Rizk Y., Pfeiffer J.A. P., Lewin J.C. Expanded access programs., compassionate drug use., and emergency use authorizations during the COVID-19 pandemic. Drug Discov Today (2021). 26:593-603. https://doi.org/10.1016/j.drudis.2020.11.025.

91. Ngo H.X., Garneau-Tsodikova S. What are the drugs of the future? Med Chem Comm (2018). 9:757-8. https://doi.org/10.1039/C8MD90019A.

92. Thomas S.J., Snowden J.A., Zeidler M.P., Danson S.J. The role of JAK/STAT signalling in the pathogenesis., prognosis and treatment of solid tumours. Br J Cancer (2015). 113:365-71. https://doi.org/10.1038/bjc.2015.233.

93. Ghoreschi K., Laurence A., O'Shea J.J. Janus kinases in immune cell signaling. Immunol Rev (2009). 1:273-87. https://doi.org/10.1111/j.1600-065X.2008.00754.x.

94. O'Shea J.J., Schwartz D.M., Villarino A.V., Gadina M., McInnes I.B., Laurence A. The JAK-STAT pathway: impact on human disease and therapeutic intervention. Annu Rev Med (2015). 66:311-328. https://doi.org/10.1146/annurev-med-051113-024537.

95. Damsky W., King B.A. JAK inhibitors in dermatology: The promise of a new drug class. J Am Acad Dermatol (2017). 76:736-44. https://doi.org/10.1016/j.jaad.2016.12.005.

96. Liu KD., Gaffen SL., Goldsmith MA. JAK/STAT signaling by cytokine receptors. Curr Opin Immunol (1998). 3:271-8. https://doi.org/10.1016/S0952-7915(98)80165-9.

97. Fragoulis G.E., McInnes I.B., Siebert S. JAK-inhibitors. New players in the field of immune-mediated diseases., beyond rheumatoid arthritis. Rheumatology (Oxford) (2019). 58(Supp_1): i43-54. https://doi.org/10.1093/rheumatology/key276.

98. Center for Drug Evaluation and Research. US Food and Drug Administration. Pharmacology review baricitinib. NDA 207-924. Apr (2017). https://www.accessdata.fda.gov/drugsatfda_docs/nda/2018/207924Orig1s000MedR.pdf

99. Schwartz D.M., Bonelli M., Gadina M., O'Shea J.J. Type I/II cytokines., JAKs., and new strategies for treating autoimmune diseases. Nat Rev Rheumatol (2016). 12(1):25-36. https://doi.org/10.1038/nrrheum.2015.167.

100. Committee for Medicinal Products for Human Use. European Medicines Agency. Assessment report Olumiant (baricitinib). EMAE/H/C/004085/0000. 15 Dec. 2016. Available from https://www.ema.europa.eu/en/documents/assessment-report/olumiant-epar-public-assessment-report_en.pdf

101. Jorgensen S. C., Tse C. L., Burry L., Dresser L. D. Baricitinib: A Review of Pharmacology., Safety., and Emerging Clinical Experience in COVID-19. Pharmacotherapy (2020). 40(8):843-856. https://doi.org/10.1002/phar.2438.

102. OLUMIANT (baricitinib) tablets., for oral use Initial U.S. Approval: (2018). https://dailymed.nlm.nih.gov/dailymed/fda/fdaDrugXsl.cfm?setid=866e9f35-9035-4581-a4b1-75a621ab55cf&type=display.

103. Dani Mann., Baricitinib shows promise in delaying progression of type 1 diabetes., (2023). https://www.diabetes.co.uk/2023/news/dec/arthritis-drug-shows-promise-in-type-1-diabetes-control.html.

104. Shi J.G., Chen X., Lee F., Emm T., Scherle P.A., Lo Y., Punwani N., Williams W.V., Yeleswaram S. The pharmacokinetics., pharmacodynamics., and safety of baricitinib., an oral JAK 1/2 inhibitor., in healthy volunteers. J Clin Pharmacol (2014). 54:1354-61. https://doi.org/10.1002/jcph.354.

105. Hsu J.Y., Mao Y.C., Liu P.Y., Lai K.L. Pharmacology and adverse events of emergency-use authorized medication in moderate to severe COVID-19. Pharmaceuticals (2021). 14:955. https://doi.org/10.3390/ph14100955.

106. Kim H., Brooks K.M., Tang C.C., Wakim P., Blake M., Brooks S. R., Montealegre Sanchez G. A., de Jesus A. A.Huang Y., Tsai W. L., Gadina M., Prakash A., Janes J. M., Zhang X., Macias W. L., Kumar P., Goldbach-Mansky R. Pharmacokinetics., pharmacodynamics., and proposed dosing of the oral JAK1 and JAK2 inhibitor baricitinib in pediatric and young adult CANDLE and SAVI patients. *Clinical Pharmacology and Therapeutics* (2018). 104(2):364-73. https://doi.org/10.1002/cpt.936.

107. Arthritis Advisory Committee Meeting FDA Briefing Document. NDA 207924. Baricitinib Janus Kinase (JAK) inhibitor for RA. Eli Lilly and Company (Lilly). 23 April (2018). Available from https://www.citizen.org/wp-content/uploads/2418.pdf.

108. Winthrop K.L. The emerging safety profile of JAK inhibitors in rheumatic disease. Nat Rev Rheumatol (2017). 13., 234-243. https://doi.org/10.1038/nrrheum.2017.23.

109. Vyas D., O'Dell K.M., Bandy J.L., Boyce E.G. Tofacitinib: The First Janus Kinase (JAK) inhibitor for the treatment of rheumatoid arthritis. Ann Pharmacother (2013). 11:1524-31. https://doi.org/10.1177/1060028013512790.

110. Qin C., Zhou L., Hu Z., Zhang S., Yang S., Tao Y., Xie C., Ma K., Shang K., Wang W., Tian D.S. Dysregulation of immune response in patients with COVID-19 in Wuhan., China. Clinical Infectious Diseases (2020). 71(15):762-8. https://doi.org/10.1093/cid/ciaa248.

111. Wang D., Hu B., Hu C., Zhu F., Liu X., Zhang J., Wang B., Xiang H., Cheng Z., Xiong Y., Zhao Y., Li Y., Wang X., Peng Z. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan., China. JAMA (2020). 323(11):1061-1069. https://doi.org/10.1001/jama.2020.1585.

112. Zheng M., Gao Y., Wang G., Song G., Liu S., Sun D., Xu Y., Tian Z. Functional exhaustion of antiviral lymphocytes in COVID-19 patients. Cell Mol Immunol (2020). 17,533-535. https://doi.org/10.1038/s41423-020-0402-2.

113. Guo T Fan Y., Chen M., Wu X., Zhang L., He T., Wang H., Wan J., Wang X., Lu Z. Cardiovascular implications of fatal outcomes of patients with coronavirus disease 2019 (COVID-19). JAMA Cardiol. (2020). 5(7):811-818. https://doi.org/10.1001/jamacardio.2020.1017.

114. Kalil A.C., Patterson T.F., Mehta A.K., Tomashek K.M., Wolfe C.R., Ghazaryan V., Marconi V.C., Ruiz-Palacios G.M., Hsieh L., Kline S., Tapson V., Iovine N.M., Jain M.K., Sweeney D.A., El Sahly H.M., Branche A.R., Regalado Pineda J., Lye D.C., Sandkovsky U., Luetkemeyer A.F., Cohen S.H., Finberg R.W., Jackson P.E., Taiwo B., Paules C. I., Arguinchona H., Erdmann N., Ahuja N., Frank M., Oh M., Kim E., Tan S.Y., Mularski R.A., Nielsen H., Ponce P.O., Taylor B. S., Larson L., Rouphael N.G., Saklawi Y., Cantos V.D., Ko E.R., Engemann J.J., Amin A.N., Watanabe M., Billings J., Elie M., Davey R. T., Burgess T .H., Ferreira J., Green M., Makowski M., Cardoso A., de Bono S., Bonnett T., Proschan M., Deye G.A., Dempsey W., Nayak S.U., Dodd L.E., Beigel J.H. Baricitinib plus Remdesivir for hospitalized adults with Covid-19. *New England Journal of Medicine* (2021). 384(9):795-807. https://doi.org/10.1056/NEJMoa2031994.

115. Sanchez G.A.M., Reinhardt A., Ramsey S., Wittkowski H., Hashkes P.J., Berkun Y., Schalm S., Murias S., Dare J.A., Brown D., Stone D. L., Gao L., Klausmeier T., Foell D., De Jesus A. A., Chapelle D. C., Kim H., Dill S., Colbert R.A., Failla L., Kost B., O'Brien M., Reynolds J.C., Folio L.R., Calvo K .R., Paul S. M., Weir N., Brofferio A., Soldatos A., Biancotto A., Cowen E.W., Digiovanna J. J., Gadina M., Lipton A.J., Hadigan C., Holland S M., Fontana J., Alawad A.S., Brown R.J., Rother K. I., Heller T., Brooks K.M., Kumar P., Brooks S. R., Waldman M.,

Singh H. K., Nickeleit V., Silk M., Prakash A., Janes J.M., Ozen S., Wakim P.G., Brogan P.A., Macias W. L., Goldbach-Mansky R. JAK1/2 inhibition with baricitinib in the treatment of autoinflammatory interferonopathies. J Clin Invest (2018). 128(7):3041-3052. https://doi.org/10.1172/JCI98814.

116. Tuttle K.R., Brosius F.C., Adler S.G., Kretzler M., Mehta R.L., Tumlin J.A., Tanaka Y., Haneda M., Liu J., Silk M.E., Cardillo T.E., Duffin K. L., Haas J.V., Macias W.L., Nunes F.P., Janes J.M. JAK1/JAK2 inhibition by baricitinib in diabetic kidney disease: results from a Phase 2 randomized controlled clinical trial. Nephrol Dial Transplant,(2018). 33(11):1950-1959. https://doi.org/10.1093/ndt/gfx377.

117. Kim H., Dill S., O'Brien M., Vian L., Li X., Manukyan M., Jain M., Adeojo L.W., George J., Perez M., Grom A.A., Sutter M., Feldman B.M., Yao L., Millwood M., Brundidge A., Pichard D.C., Cowen E .W., Shi Y., Lu S., Tsai W. L., Gadina M., Rider L.G., Colbert R .A. Janus kinase (JAK) inhibition with baricitinib in refractory juvenile dermatomyositis. Ann Rheum Dis. (2021). https://doi.org/10.1136/annrheumdis-2020-218690

118. Guttman-Yassky E., Silverberg J.I., Nemoto O., Forman S.B., Wilke A., Prescilla R., de la Peña A., Nunes F.P., Janes J., Gamalo M., Donley D., Paik J., DeLozier A.M., Nickoloff B. J., Simpson E.L. Baricitinib in adult patients with moderate-to-severe atopic dermatitis: a phase 2 parallel., double-blinded., randomized placebo-controlled multiple-dose study. J Am Acad Dermatol (2019). 80(4):913-921.e9. https://doi.org/10.1016/j.jaad.2018.01.018.

119. Rodrigues M.A., Torres T. JAK/STAT inhibitors for the treatment of atopic dermatitis. J Dermatolog Treat (2020). 31(1):33-40. https://doi.org/10.1080/09546634.2019.1577549

120. Reich K., Kabashima K., Peris K., Silverberg J. I., Eichenfield L.F., Bieber T., Kaszuba A., Kolodsick J., Yang F. E., Gamalo Brinker D. R., Delozier A. M., Janes J .M., Nunes F.P., Thyssen J.P., Simpson E. L. Efficacy and safety of Baricitinib combined with topical corticosteroids for treatment of moderate to severe atopic dermatitis: a randomized clinical trial. JAMA Dermatol. (2020).156(12):1333-1343. https://doi.org/10.1001/jamadermatol.2020.3260.

121. Mumford B.P., Gibson A., Chong A.H. Repigmentation of vitiligo with oral baricitinib. Australas J Dermatol (2020). 61(4):374-376. https://doi.org/10.1111/ajd.13348.

122. Qi F., Liu F., Gao L. Janus kinase inhibitors in the treatment of vitiligo: a review. Front Immunol (2021). 12:790125. https://doi.org/10.3389/fimmu.2021.790125.

123. Strazzulla L.C., Wang E.H.C., Avila L., Lo Sicco K., Brinster N., Christiano A. M., Shapiro J. Alopecia areata: disease characteristics., clinical evaluation., and new perspectives on pathogenesis. J Am Acad Dermatol (2018). 78(1):1-12. https://doi.org/10.1016/j.jaad.2017.04.1141.

124. Messenger A.G., McKillop J., Farrant P., McDonagh A.J., Sladden M. British Association of Dermatologists guidelines for the management of Alopecia Areata 2012. Br J Dermatol (2012). 166(5):916-926. https://doi.org/10.1111/j.1365-2133.2012.10955.x.

125. King B., Ko J., Forman S., Ohyama M., Mesinkovska N., Yu G., McCollam J., Gamalo M., Janes J., Edson-Heredia E., Holzwarth K., Dutronc Y. Efficacy and safety of the oral Janus kinase inhibitor baricitinib in the treatment of adults with alopecia areata: phase 2 results from a randomized controlled study. J Am Acad Dermatol (2021). 85(4):847-853. https://doi.org/10.1016/j.jaad.2021.05.050

126. Papp K. A., Menter M. A., Raman M., Disch D., Schlichting D. E., Gaich C., Macias W., Zhang X., Janes J. M. A randomized phase 2b trial of baricitinib., an oral Janus kinase (JAK) 1/JAK2 inhibitor., in patients with moderate-to-severe psoriasis., British Journal of Dermatology (2016). 174:1266-76. https://doi.org/10.1111/bjd.14403.

127. Menter A., Disch D., Clemens J., Janes J., Papp K., Macias W. A phase 2b trial of baricitinib., an oral JAK inhibitor., in patients with moderate to severe psoriasis. Journal of the American Academy of Dermatology (2014). 70(5): AB162. https://doi.org/10.1016/j.jaad.2014.01.676.

128. Hiraganahalli Bhaskarmurthy D., Evan Prince S. Effect of Baricitinib on TPA-induced psoriasis like skin inflammation. Life Sciences (2021). 15:279:119655 https://doi.org/10.1016/j.lfs.2021.119655.

129. Di Domizio J., Castagna J., Algros M.P., Prati C., Conrad C., Gilliet M., Wendling D., Aubin F. Baricitinib-induced paradoxical psoriasis., Journal of the European Academy of Dermatology and Venereology (2020). 34 (8): e391–e393. https://doi.org/10.1111/jdv.16293.

130. Atzeni F., Talotta R., Nucera V., Marino F., Gerratana E., Sangari D., Masala I.F., Sarzi-Puttini P. Adverse events., clinical considerations and management recommendations in rheumatoid arthritis patients treated with JAK inhibitors. Expert Rev Clin Immunol (2018). 14(11):945-956. https://doi.org/10.1080/1744666X.2018.1504678.

131. Dai Z., Chen J., Chang Y., Christiano A.M. Selective inhibition of JAK3 signaling is sufficient to reverse alopecia areata. JCI Insight (2021). 6:7. https://doi.org/10.1172/jci.insight.142205.

132. Xu H., Jesson M.I., Seneviratne UI., Lin T.H., Sharif M.N., Xue L., Nguyen C., Everley R A., Trujillo J.I., Johnson D.S., Point G.R., Thorarensen A., Kilty I., Telliez J. B. PF-06651600, a dual JAK3/TEC family kinase inhibitor. ACS Chem Biol (2019). 14(6):1235-1242. https://doi.org/10.1021/acschembio.9b00188.

133. Opzelura for AA: https://www.opzelura.com/.

134.Opzelura for vitiligo: https://www.opzelura.com/vitiligo/.

135. https://cima.aemps.es/cima/dochtml/p/1231726001/P_1231726001.html.

136. https://www.ema.europa.eu/en/medicines/human/EPAR/opzelura.

137. Winthrop K.L., Silverfield J., Racewicz A., Neal J., Lee E.B., Hrycaj P., Gomez-Reino J., Soma K., Mebus C., Wilkinson B., Hodge J., Fan H., Wang T., Bingham C.O. The effect of tofacitinib on pneumococcal and influenza vaccine responses in rheumatoid arthritis. Ann Rheum Dis (2016). 75(4):687-695. https://doi.org/10.1136/annrheumdis-2014-207191.

138. Groeber F., Holeiter M., Hampel M., Hinderer S., Schenke-Layland K. Skin tissue engineering—in vivo and in vitro applications. Adv. Drug Deliv. Rev (2011). 63., 352-366. https://doi.org/10.1016/j.addr.2011.01.005.

139. Feldmann R.J., Maibach H.I. Absorption of Some Organic Compounds through the Skin in Man.J. Investig. Derm (1970). 54., 399-404. https://doi.org/10.1111/1523-1747.ep12259184.

140. Blank I.H. Factors Which Influence the Water Content of the Stratum Corneum. J. Investig. Derm (1952). 18.,433-440. https://doi.org/10.1038/jid.1952.52.

141. Vinson L.J., Singer E.J., Koehler W.R., Lehman M.D., Masurat T. The nature of the epidermal barrier and some factors influencing skin permeability. Toxicol. Appl. Pharm (1965). 7., 7-19. https://doi.org/10.1016/0041-008X(65)90107-9.

142. Elias P.M., Friend D. S. The permeability barrier in mammalian epidermis. J. Cell Biol (1975). 65., 180-191. https://doi.org/10.1083/jcb.65.1.180.

143. Sweeney T.M., Downing D.T. The Role of Lipids in the Epidermal Barrier to Water Diffusion. J. Investig. Derm (1970). 55., 135-140. https://doi.org/10.1111/1523-1747.ep12291678.

144. Anissimov Y.G., Jepps O.G., Dancik Y., Roberts M.S. Mathematical and pharmacokinetic modelling of epidermal and dermal transport processes. Adv. Drug Deliv. Rev (2013). 65., 169-190. https://doi.org/10.1016/j.addr.2012.04.009.

145. Bo Forslind. A domain mosaic model of the skin barrier. Acta Derm. Venereol (1994). 74., 1-6. https://doi.org/10.2340/00015555741214.

146. Godin B., Touitou E. Transdermal skin delivery: Predictions for humans from in vivo., ex vivo and animal models. Adv. Drug Deliv. Rev (2007). 59., 1152-1161. https://doi.org/10.1016/j.addr.2007.07.004.

147. Gravitz L. Skin. Nature [Internet]. 21 November 2018., 563(7732): S83-S83. Disponible a: http://www.nature.com/articles/d41586-018-07428-4.

148. Buendía Eisman A., Mazuecos Blanca J., Camacho Martínez F.M. Manual de Dermatología- Anatomía y fisología de la piel. (2018). pages 2-27. ISBN Volumen I: 978-84-7885-628-2. ISBN Obra completa: 978-84-7885-627-5. ISBN Volumen II:978-84-7885-629-9. https://www.berri.es/pdf/MANUAL%20DE%20DERMATOLOGIA%E2%80%9A%202%20Vols.%20(Tapa%20Dura)/9788478856282.

149. FortoulvanderGoes T. Histología y biología celular [Book]. 3e Edition., AccessMedicina., Editor: McGraw-Hill Interamericana Editores (2017). ISBN 10: 6071514088 / ISBN 13: 9786071514080. Disponible a: https://accessmedicina-mhmedical-com.sire.ub.edu/content.aspx?bookid=1995&sectionid=150301510#1138472886.

150. Puig Sanz L. Capítulo 1., Anatomía y fisiología de la piel humana. En: Ferrándiz Dermatología clínica [Book]. Fifth Edition. Spain: Elsevier (2019). P. 1.,10. e1-10.,10. e1. Disponible a: https://www.clinicalkey.com/student/content/toc/3-s2.0-C2017001858X.

151. Hadgraft., J. Skin deep. Eur. J. Pharm Bio pharm (2004). 58(2)., 291-299. https://doi.org/10.1016/j.ejpb.2004.03.002.

152. Pandya B., Mayhew J. Ultrastructure of Skin., TeachMe Anatomy (2022). web page: https://teachmeanatomy.info/the-basics/ultrastructure/skin/.

153. FerrándizForaster C., BielsaMarsol MaI. Ferrándiz Dermatología clínica., Elsevier., 5th edition (2019). ISBN-13: 9788491132646. Disponible a: https://www.clinicalkey.com/student/content/toc/3-s2.0-C2017001858X.

154. FortoulvanderGoes T. Histología y biología celular. 3a edition Fortoul van der Goes T., editor. México., D.F: McGraw-Hill (2019). Book.

155. Ruela A.L.M., Perissinato A.G. Lino M.E.D.S., Mudrik P.S., Pereira G.R. Evaluation of skin absorption of drugs from topical and transdermal formulations. Braz. J. Pharm. Sci (2016). 52., 527-544. https://doi.org/10.1590/s1984-82502016000300018.

156. Shah V.P., Yacobi A., Radulescu F.S., Miron D.S., Lane., M.E. A science based approach to topical drug classification system (TCS). International Journal of Pharmaceutics (2015). 491., 21-25. https://doi.org/10.1016/j.ijpharm.2015.06.011.

157. Kalia Y.N., Guy R.H. Modeling transdermal drug release. Advanced Drug Delivery Reviews. Advanced Drug Delivery Reviews (2001). 48., 159-172. https://doi.org/10.1016/S0169-409X(01)00113-2.

158. Machado A.C.H.R., Lopes P.S., Ra_er C.P., Haridass I.N., Roberts M., Grice J., Leite-Silva V.R. Skin Penetration. In Cosmetic Science and Technology., Elsevier: Amsterdam., The

Netherlands (2017). PP. 741-755.ISBN 978-0-12-802005-0. https://doi.org/10.1016/B978-0-12-802005-0.00046-X.

159. Moser K., Kriwet K., Naik A., Kalia Y.N., Guy R.H. Passive skin penetration enhancement and its quantification in vitro. European Journal of Pharmaceutics and Biopharmaceutics. Biopharm (2001). 10., 103-112. https://doi.org/10.1016/S0939-6411(01)00166-7.

160. Delgado-Charro M.B., Guy R.H. Efective use of transdermal drug delivery in children. Adv. Drug Deliv. Rev (2014). 73., 63-82. https://doi.org/10.1016/j.addr.2013.11.014.

161. Dragicevic N., Maibach H.I. Percutaneous Penetration Enhancers Drug Penetration Into/Through the Skin., Springer: Berlin/Heidelberg., Germany (2017). ISBN 978-3-662-53268-3. Book. https://doi.org/10.1007/978-3-662-53270-6.

162. Machado M., Hadgraft J., Lane M.E. Assessment of the variation of skin barrier function with anatomic site., age., gender and ethnicity: Assessment of the variation of skin barrier function. International Journal of Cosmetic Science (2010). 32., 397-409. https://doi.org/10.1111/j.1468-2494.2010.00587.x.

163. Waller J.M., Maibach H.I. Age and skin structure and function., a quantitative approach (I): Blood flow., pH., thickness., and ultrasound echogenicity. Skin Research and Technology (2005). 11., 221-235. https://doi.org/10.1111/j.0909-725X.2005.00151.x.

164. Behl C.R., Flynn G.L., Barrett M., Walters K.A., Linn E.E., Mohamed Z., Kurihara T., Ho N.F.H., Higuchi W.I., Pierson C.L. Permeability of thermally damaged skin II: Immediate influences of branding at 60 ^{0}C on hairless mouse skin permeability. Burns (1981). 7., 389-399. https://doi.org/10.1016/0305-4179(81)90125-X.

165. Behl C.R., Flynn G.L., Kurihara T., Smith W., Gatmaitan O., Higuchi W.I., Ho N.F.H., Pierson C.L. Permeability of Thermally Damaged Skin: I. Immediate Influences of 60^{0} C Scalding on Hairless Mouse Skin. Journal of Investigative Dermatology (1980). 75., 340-345. https://doi.org/10.1111/1523-1747.ep12531096.

166. Flaten G.E., Palac Z., Engesland A., Filipovi´c-Gr˘ci´c J., Vani´c Ž., Škalko-Basnet N. In vitro skin models as a tool in optimization of drug formulation. European Journal of Pharmaceutical Sciences (2015). 75., 10–24. https://doi.org/10.1016/j.ejps.2015.02.018.

167. Zsikó S., Csányi E., Kovács A., Budai-Szűcs M., Gácsi A., Berkó S. Methods to evaluate skin penetration in vitro. Scientia Pharmaceutica (2019). https://doi.org/10.3390/scipharm87030019.

168. Wang S., Sun M., Ping Q. Enhancing effect of Labrafac Lipophile WL 1349 on oral bioavailability of hydroxy safflor yellow A in rats. International Journal of Pharmaceutics (2008). 24;358(1-2):198-204. http://dx.doi.org/10.1016/j.ijpharm.2008.03.006.

169. United States Pharmacopeial Convention., USP-NF (2013). National Formulary 31st edition monograph entitled Diethylene Glycol Monoethyl Ether. United Book Press., Baltimore., MD.

170. Rowe R.C., Sheskey P.J., Cook W.G., Fenton M.E. Diethylene glycol monoethyl ether. In: Pharmaceutical Press and the American Pharmacists Association (Ed.) (2012). Handbook of Pharmaceutical Excipients., seventh ed., Washington., DC., PP. 256-258.

171. Sullivan D.W., Gad S.C., Julien M. A review of the nonclinical safety of Transcutol®., a highly purified form of diethylene glycol monoethyl ether (DEGEE) used as a pharmaceutical excipient. Food Chem Toxicol. (2014). 72:40-50. https://doi.org/10.1016/j.fct.2014.06.028.

172. Sanidad D. E., Social Y. B. Draft Guideline on quality and equivalence of topical products, EMA European Medicines Agency Scientific Committee of Consumer Safefty. Ema/Chmp/Qwp/708282/2018 (2019) https://www.ema.europa.eu/en/documents/scientific-guideline/draft-guideline-quality-and-equivalence-topical-products_en.pdf. (annex 1., page 25).

173. In Vitro Release Test Studies for Topical Drug Products Submitted in ANDAs Guidance for Industry, U.S. Department of Health and Human Services Food and Drug Administration, Center for Drug Evaluation and Research (CDER)October 2022, Generic Drugs. https://www.fda.gov/media/162476/download.

174. Bruschi M. L. Strategies to Modify the Drug Release from Pharmaceutical Systems, Chapture 5-Mathematical models of drug release, Strategies to Modify the Drug Release from Pharmaceutical Systems (2015). P. 63-86, ISBN 9780081000922, https://doi.org/10.1016/B978-0-08-100092-2.00005-9.

175. Trucillo P. Drug Carriers: A Review on the Most Used Mathematical Models for Drug Release, Processes (2022). 10, 1094, https://doi.org/10.3390/pr10061094.

176. OECD. guidelines for the testing of chemicals. 427. Skin absorption: in vivo method., OECD: Paris., France., (2004).

177. Test No. 428: Skin Absorption: In Vitro Method. OECD guideline for the testing of chemicals., OECD: Paris., France., (2004).

178. OECD. Guidance Document for the Conduct of Skin Absorption Studies., OECD Series on Testing and Assessment., OECD: Paris., France., (2004). ISBN 978-92-64-07879-6.

179. Kielhorn., J. International Programme on Chemical Safety Dermal Absorption., Environmental Health Criteria., WHO: Geneva., Switzerland., (2006). ISBN 978-92-4-157235-4.

180. European Centre for Ecotoxicology and Toxicology of Chemicals. Percutaneous absorption., Monograph., No. 20., European Centre for Ecotoxicology and Toxicology of Chemicals: Bruxelles., Belgium (1993). ISSN 0773-6347-20.

181. EPAU S. Environmental Protection Agency (EPA). Dermal Exposure Assessment: A Summary of EPA Approaches. Available online: https://assessments.epa.gov/risk/document/&deid%3D183584.

http://www.epa.gov/ncea. (accessed on 1 April 2019).

182. Buist H., Craig P., Dewhurst I., Hougaard Bennekou S., Kneuer C., Machera K., Pieper C., Court Marques D., Guillot G., Ruffo F., Chiusolo A. Guidance on dermal absorption. EFSA Journal (2017). 15., e04873.http://dx.doi.org/10.2903/j.efsa.2017.4873.

183. Dumont C., Prieto P., Asturiol D., Worth A. Review of the Availability of In Vitro and In Silico Methods for Assessing Dermal Bioavailability. Applied In Vitro Toxicology. (2015). 1., 147-164. http://dx.doi.org/10.1089/aivt.2015.0003.

184. Validation of analytical procedures: text and methodology Q2 (R1., in ICH Guideline., Harmonized Tripartite)., IFPMA: Geneva (2005).

185. Baldi A., Panwar M.S., Swapnil G. Development and validation of spectrophotometric method for determination of ropinirole hydrochloride in bulk and formulation., Inventi Rapid: Pharm Analysis & Quality Assurance (2015). 1:1-4.

186. Ibrahim F., El-Enany N.M., Shalan S., Abo Shabana R.A. Validated sensitive spectrophotometric methods for determination of carvedilol and nebivolol hcl in dosage forms., J. Adv. Chem (2014). 10., 2796-2813. http://dx.doi.org/10.24297/jac. v10i6.6667.

187. A. El Yazbi F., E. Mahrous M., H. Hammud H., M. Sonji G., M. Sonji N. Kinetic Spectrophotometric Determination of Betaxolol., Clopidogrel and Imidapril in Pharmaceutical Preparations., *Current Analytical Chemistry* (2010). 6., 228-236., http://dx.doi.org/10.2174/157341110791517007

188. Belal F., Al-Shaboury S., Al-Tamrah A.S. Spectrofluorometric determination of labetolol in pharmaceutical preparations and spiked human urine through the formation of coumarin derivative., J. Pharm. Biomed. Anal (2002). 30., 1191-1196. http://dx.doi.org/10.1016/S0731-7085(02)00471-5.

189. Tabrizi A.B. A simple spectrofluorimetric method for determination of piroxicam and propranolol in pharmaceutical preparations., Journal of Food and Drug Analysis., Vol. 15., No. 3(2007). P. 242-248. http://dx.doi.org/10.38212/2224-6614.2414.

190. Mohammadi-Meyabadi R., Beirampour N., Garrós. N., Alvarado H.L., Limón D., Silva-Abreu M., Calpena A.C., Mallandrich M. Assessing the Solubility of Baricitinib and Drug

Uptake in Different Tissues Using Absorption and Fluorescence Spectroscopies. Pharmaceutics (2022). 14., 2714. http://dx.doi.org/10.3390/pharmaceutics14122714.

191. Gandhi S.V., Kapoor B.G. Development and Validation of UV Spectroscopic Method for Estimation of Baricitinib., Journal of Drug Delivery and Therapeutics (2019). 9(4-s):488-491. http://dx.doi.org/10.22270/jddt.v9i4-s.3230.

192. Thakur S., Bajwa N., Baldi A. NEW ANALYTICAL METHODS FOR ESTIMATION OF ARTEETHER BY UV AND FLUORESCENCE SPECTROPHOTOMETRY: DEVELOPMENT AND VALIDATION., Journal of Drug Delivery & Therapeutics (2018). 8(5):151-158. https://dx.doi.org/10.22270/jddt.v8i5.1797.

193. Mazzarino L., Bellettini I.C., Minatti E., Lemos-Senna E. Development and validation of a fluorimetric method to determine curcumin in lipid and polymeric nanocapsule suspensions. Braz J Pharm Sci (2010). 46:219-226. https://dx.doi.org/10.1590/S1984-82502010000200008.

194. Bhagav P., Deshpande P., Pandey S., Chandran S., Development and validation of stability indicating UV spectrophotometric method for the estimation of brimonidine tartrate in pure form., formulations and pre-formulation studies. Der Pharm Lett (2010). 2(3):106-122.

195. Ayad M.M., Abdellatef H.E., El-Henawee M.M., El-Sayed H.M. Spectrophotometric and spectrofluorimetric methods for analysis of acyclovir and acebutolol hydrochloride. Spectrochim (2007). Acta A Mol Biomol Spectrosc 66:106-110. https://doi.org/10.1016/j.saa.2006.02.031.

196. Aktas E.S., Ersoy L., Sagirli O. A new spectrofluorimetric method for the determination of lisinopril in tablets. Farmaco (2003). 58: 165-168. https://doi.org/10.1016/S0014-827X(02)00013-7.

197. Gandhi S. V., Kapoor B. G. Development and Validation of UV Spectroscopic Method for Estimation of Baricitinib. Journal of Drug Delivery & Therapeutics. (2019). 9(4-s):488-491. http://dx.doi.org/10.22270/jddt.v9i4-s.3230.

198. J.N Miller., J.C Miller. Statistics and Chemometrics for Analytical Chemistry., Prentice Hall., 4th ed., (2005). book.

199. Nițescu D., Mușetescu A., Nițescu M., Costescu M., Coman O. Experimental research in topical psoriasis therapy (Review)Experimental and Therapeutic Medicine (2021). https://doi.org/10.3892/etm.2021.10403.

200. Soufizadeh P., Mansouri V., Ahmadbeigi N. A review of animal models utilized in preclinical studies of approved gene therapy products: trends and insights. Lab Anim Res (2024). 40.,17. https://doi.org/10.1186/s42826-024-00195-6.

201. Boehncke W., Schön M. Animal models of psoriasis. Clinics in Dermatology (2007). 25 6., 596-605. https://doi.org/10.1016/J.CLINDERMATOL.2007.08.014.

202. Rodríguez-Martínez S., Cancino-Diaz J.C., Martínez-Torrez I.M., Pérez-Tapia S.M., Cancino-Diaz M. Psoriatic Animal Models Developed for the Study of the Disease. An Interdisciplinary Approach to Psoriasis (2017). https://doi.org/10.5772/INTECHOPEN.68305.

203. Jabeen M., Boisgard A.S., Danoy A., El Kholti N., Salvi J.P., Boulieu R., Fromy B., Verrier B., Lamrayah M. Advanced Characterization of Imiquimod-Induced Psoriasis-Like Mouse Model. Pharmaceutics (2020).12.,789. https://doi.org/10.3390/pharmaceutics12090789.

204. Braun-Falco O. THE HISTOCHEMISTRY OF PSORIASIS. Annals of the New York Academy of Sciences (1958). 73. https://doi.org/10.1111/j.1749-6632.1959.tb40870.x.

205. EMA guideline: 22 October 2015., EMA/CHMP/SWP/2145/2000 Rev. 1., Corr. 1*Committee for Medicinal Products for Human Use (CHMP). https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-non-clinical-local-tolerance-testing-medicinal-products_en.pdf.

206. Lau E. Handbook of Modern Pharmaceutical Analysis: Preformulation studies. Separation Science and Technology (2001). Book Chapter, 5 - Preformulation Studies, Edward Lau, P. 173-233. https://doi.org/10.1016/S0149-6395(01)80007-6.

https://www.sciencedirect.com/science/article/abs/pii/S0149639501800076.

207. Kim J.Y., Kim S., Papp M., Park K., Pinal R. Hydrotropic solubilization of poorly water-soluble drugs. Journal of Pharmaceutical Sciences (2010). https://doi.org/10.1002/jps.22241.

https://jpharmsci.org/article/S0022-3549(15)32499-0/fulltext.

208. Savjani K.T., Gajjar A.K., Savjani J.K. Drug Solubility: Importance and Enhancement Techniques. ISRN Pharmaceutics (2012). https://doi.org/10.5402/2012/195727.

209. Miyako Y., Khalef N., Matsuzaki K., Pinal R. Solubility enhancement of hydrophobic compounds by cosolvents: Role of solute hydrophobicity on the solubilization effect. International Journal of Pharmaceutics (2010). P. 48-54. https://doi.org/10.1016/j.ijpharm.2010.03.059.

210. Choi S.A., Park E.J., Lee J.H., Min K. A S T., Jang D.J., Maeng H.J., Jin S. G., Cho K. H. Preparation and Characterization of Pazopanib Hydrochloride-Loaded Four-Component Self-Nanoemulsifying Drug Delivery Systems Preconcentrate for Enhanced Solubility and

Dissolution. Pharmaceutics (2022). 14(9), 1875; https://doi.org/10.3390/pharmaceutics14091875.

211. Committee for Medicinal Products for Human Use (CHMP). European Medicines Agency Guideline on Bioanalytical Method Validation; CHMP: London, UK, (2011). P. 1-23.https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-bioanalytical-method-validation_en.pdf.

212. Asociación Española de Farmacéuticos de la Industria (Ed.). Validación de Métodos Analíticos; Asociación Española de Farmacéuticos de la Industria: Madrid, Spain, (2001). ISBN 8489602336. https://www.academia.edu/10365264/Validacion_de_Metodos_Analiticos_Asociacion_Espanola_de_Farmaceuticos_de_la_Industria.

213. Sørensen D. H., Christensen N. P. A., Skibsted E., Rantanen J., Rinnan Å. In-line Fluorescence Spectroscopy for Quantification of Low Amount of Active Pharmaceutical Ingredient. Journal of Pharmaceutical Sciences (2022). P. 2406-2410. https://doi.org/10.1016/j.xphs.2022.06.008. https://www.sciencedirect.com/science/article/pii/S0022354922002519.

214. Assalem N., Abd-allah H., Ragaie M.H., Ahmed S.S, Elmowafy E. Therapeutic potential of limonene-based syringic acid nanoemulsion: Enhanced ex-vivo cutaneous deposition and clinical anti-psoriatic efficacy, International Journal of Pharmaceutics, (2024). 660: 124376. https://doi.org/10.1016/j.ijpharm.2024.124376.

215. Sosa L., Calpena A., Silva-Abreu M., Espinoza L., Rincón M., Bozal N., Domenech O., Rodríguez-Lagunas M., Clares B. Thermoreversible Gel-Loaded Amphotericin B for the Treatment of Dermal and Vaginal Candidiasis. Pharmaceutics (2019). 11, 312. https://doi.org/10.3390/pharmaceutics11070312.

216. Welin-Berger K., Neelissen J., Bergenståhl B. In vitro permeation profile of a local anaesthetic compound from topical formulations with different rheological behaviour - Verified by in vivo efficacy data.European Journal of Pharmaceutical Sciences (2001). 14(3):229-36. https://doi.org/10.1016/S0928-0987(01)00181-6.

217. Asghar L. F. A. Chandran S. Design and evaluation of matrix base with sigmoidal release profile for colon-specific delivery using a combination of Eudragit and non-ionic cellulose ether polymers. Drug Delivery and Translational Research (2011). 1(2):132-46. https://doi.org/10.1007/s13346-011-0016-4.

218. Hagen M., Baker M. Skin penetration and tissue permeation after topical administration of diclofenac. Current Medical Research and Opinion (2017). 33(9):1623-1634. https://doi.org/10.1080/03007995.2017.1352497.

219. Marwah H., Garg T., Goyal A.K., Rath G. Permeation enhancer strategies in transdermal drug delivery. Drug Delivery (2016). 23(2):564-78. https://doi.org/10.3109/10717544.2014.935532.

220. Zhao X., Sheng X. Y., Payne C. D., Zhang X., Wang F., Cui Y. M. Pharmacokinetics, Safety, and Tolerability of Single- and Multiple-Dose Once-Daily Baricitinib in Healthy Chinese Subjects: A Randomized Placebo-Controlled Study. Clinical Pharmacology in Drug Development (2020). 9(8):952-960. https://doi.org/10.1002/cpdd.868.

221. Balak D., Hajdarbegovic E. Drug-induced psoriasis: clinical perspectives. Psoriasis: Targets and Therapy (2017). 7:87-94. P. 87-94. https://doi.org/10.2147/PTT.S126727.

222. Lodén M. Effect of moisturizers on epidermal barrier function Clinics in Dermatology (2012). 30(3):286-96. P.286-296. https://doi.org/10.1016/j.clindermatol.2011.08.015.

223. Pober J.S., Sessa W.C. Inflammation, and the blood microvascular system.Cold Spring Harbor Perspectives in Biology (2015). 7(1): a016345. https://doi.org/10.1101/cshperspect.a016345.

224. Kurebayashi A.K., Phan K., Abdoh A., Andreo-Filho N., Lopes P.S., Mohammed Y., Leite-Silva V.R. Strategic approaches in formulation development for atopic dermatitis. Cosmetics (2024). 11(4)113. https://doi.org/10.3390/cosmetics11040113.