Master Degree
in Pharmaceutical Technology

Rational design of topical formulations for hand-foot syndrome management

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2019
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November 2019

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Acknowledgments

Throughout the writing of this dissertation I have received a great deal of support and assistance.

I would first like to thank my supervisor, Prof. Dr. Isabel Almeida, whose expertise was invaluable in the formulating of the research topic and methodology in particular. Thank you for provided me with the tools, the stimulus and the friendly words that I needed to choose the right direction.

I would like to thank to Prof. Dr. Vera Almeida for the support in psychosocial approach presented in this dissertation.

I would particularly like to single out my supervisor at Faculty of Pharmacy and Centre for Neuroscience and Cell Biology of University of Coimbra, Prof. Dr. Maria Teresa Cruz Rosete, I want to thank you for your excellent cooperation and for all of the opportunities given. I also like to thank to the researchers Isabel Ferreira and Ana Silva.

I would like to acknowledge Marta Ferreira, a Master student in the Pharmaceutical Technology Laboratory, for her wonderful and valuable collaboration which helped to successfully complete my dissertation.

Additionally, I am particularly grateful for the assistance given by colleagues from Product Quality and Compliance department at Bluepharma, mainly to Ana Lemos and Catarina Dias for their support and motivation. A special mention to Dr. Teresa Murta for her encouragement.

Finally, last but by no means least, I would like to thank Marco and my family for their wise counsel, sympathetic ear and for their love and support. You are always there for me.
Abstract

One of the purposes of this work is to design an emulgel containing diclofenac sodium and carvedilol with the aim of minimize the HFS-associated inflammation, burning and erythema caused by capecitabine treatment. Additionally, non-irritating and moisturizing ingredients were included to help to minimize xerosis and desquamation. Since these formulations are intended to be applied on the hands, special consideration was given to the sensory properties such as low stickiness and low residue, to improve patient’s satisfaction and adherence. Furthermore, a cosmetic product with enoxolone and calendula was also formulated in order to prevent and minimize the HFS symptoms like xerosis, erythema and desquamation.

The anti-inflammatory effect of diclofenac, enoxolone and calendula oil was assessed by its availability to reduce NO production in LPS-induced inflammatory in mouse leukemic monocyte macrophage cells (RAW 264.7). Additionally, a cellular viability test (AlamarBlue®) was performed to evaluate the tested compounds safety profile. The preparations, obtained from a polyacrilic acid polymer hydrogel, were characterized. The textural analysis was performed in the compression mode in a texturometer by carrying out a spreadability test. Measurements were performed in triplicate at 20°C. The parameter negative area (correlated with adhesiveness) was calculated from the texturogram. Evaporation rate and residue were evaluated after application on polymethyl methacrylate plates (1.5 mg/cm²). Petrolatum was similarly evaluated for comparison purposes. Physical stability was evaluated by centrifugation. The tested compounds effectively possess good potential as anti-inflammatory agents and it was showed that they present a safety profile, since no alterations on this type of cell viability were detected.

The optimized drug product formulation presented balanced firmness and adhesiveness results leading to a product with desired characteristics to improve patient’s satisfaction and adherence.

The cosmetic product formulation also has good characteristics but a fourth replicate should be prepared to ensure the suitability/reproducibility of preparation method and to confirm the observed characteristics.

This approach is crucial to promote the maintenance of the anti-cancer treatment, reducing the probability of cessation of therapy or a dose reduction. Considering the referred results both emulgel formulations can have a positive effect on HFS symptoms reduction and adherence but should be further confirmed through in vivo/clinical trials.

Keywords: Hand foot syndrome | capecitabine | topical product design | management of cutaneous side effects.
Resumo
Um dos objectivos desta dissertação é formular um emulgel contendo diclofenac e carvedilol com o intuito de minimizar a inflamação, sensação de ardor e eritema associados ao Síndrome mão-pé causado pelo tratamento oncológico com capecitabina. Excipientes não irritantes e hidratantes foram também incluídos para ajudar a minimizar a xerose e a descamação.
Um produto cosmético com enoxolona e óleo de calêndula como ativos foi também formulado visando a prevenção dos sintomas de Síndrome mão-pé, tais como xerose, eritema e descamação.
Estas formulações deverão ser aplicadas, principalmente, nas mãos e pés, pelo que foi dada especial atenção às propriedades sensoriais, tais como baixa viscosidade e baixo resíduo, de modo a melhorar a satisfação e a adesão.
Foi avaliada a actividade anti-inflamatória do diclofenac, da enoxolona e do óleo de calêndula através da sua capacidade de diminuir a produção de NO, induzida pela presença de LPS em macrófagos de rato (RAW 264.7). Foi também efectuado um teste de viabilidade celular (AlamarBlue®) por forma a avaliar o perfil de segurança dos compostos testados. Os resultados obtidos comprovam que estes compostos possuem potencial como agentes anti-inflamatórios e são considerados seguros e não citotóxicos.
As preparações obtidas foram caracteizadas. A análise da textura foi realizada com recurso a texturômetro através da realização de um teste de espalhabilidade, em três medições a 20 °C. A taxa de evaporação e o resíduo foram avaliados após aplicação em placas de polimetilmetacrilato (1,5 mg / cm²), bem com a vaselina para fins de comparação. A estabilidade física foi avaliada por centrifugação.
A formulação otimizada do medicamento apresentou resultados equilibrados entre firmeza e adesividade, demonstrando ser um produto com as características desejadas e com potencial para aumentar a satisfação e a adesão do paciente.
O cosmético apresenta igualmente boas características, mas deve ser preparada uma quarta formulação de modo a garantir a adequação / reprodutibilidade do método de preparação e confirmar as características observadas.
Esta abordagem é crucial para promover a manutenção do tratamento do cancro, reduzindo a probabilidade de cessação da terapia ou de redução da dose.
Considerando os resultados referidos, ambas as formulações de emulgel podem ter um efeito positivo na prevenção e na redução dos sintomas de HFS. No entanto, estes resultados devem ser confirmados através de ensaios clínicos in vivo / clínicos.

Palavras chave: Síndrome mão-pé | capecitabina | design de um produto de uso tópico | gestão de efeitos adversos cutâneos
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List of Abbreviation

QoL - Quality of Life
HFS - Hand-Foot Syndrome
HFSR - Hand-Foot Syndrome Reaction
cSCC - Squamous cell skin cancer
BRAF - Human gene that encodes a protein called B-Raf
VEGFR - Vascular endothelial growth factor receptor
DNA - Deoxyribonucleic acid
PLD - Pegylated liposomal doxorubicin
NCI-CTCAE v5.0 - National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0
ADL - Activities of Daily Living
5-FU - 5- fluorouracil
5'−DFCR - 5'-deoxy-5-fluorocytidine
CES2 - carboxylesterase 2
5'−DFUR - 5'-deoxy-5-fluorouridine
CD - cytidine deaminase
TP - thymidine phosphorylase
COX - cyclooxygenase
NF-κB - Nuclear factor kappa-light-chain-enhancer of activated B cells
IL - Interleukin
TNF - Tumor necrosis factor
TGF - Transforming growth factor
MMP2 - Matrix metallopeptidase 2
NSAID - Nonsteroidal anti-inflammatory drug
βAR - beta-adrenergic receptor
αAR - alpha-adrenergic receptor
BCS - Biopharmaceutical Classification System
WHO - World Health Organization
NE - Norepinephrine
E - Epinephrine
LTC4 - Leukotriene C4
PGE2 - Prostaglandin E2
IκB-α - NFKB inhibitor alpha
ROS - Reactive oxygen species
PI3K - Phosphoinositide-3-kinase
TxA2 - Thromboxane A2
GA - Glycyrrhetinic acid
RNS - Reactive nitrogen species
ICH - International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
QTPP - Quality target product profile
QC - Quality control
CQA - Critical quality attribute
CMA - Critical material attribute
CPP - Critical process parameter
NOS - Nitric oxide synthase
NO - Nitric oxide
NO₂ - Nitrogen dioxide
ATCC TIB-71 - American Type Culture Collection
DMEM - Dulbecco’s Modified Eagle Medium
CO₂ - Carbon dioxide
LPS - Lipopolysaccharides
NED - N-(1-naphthyl) ethylenediamine dihydrochloride
H₃PO₄ - Phosphoric acid
DMSO - Dimethyl sulfoxide
RSD - Relative standard deviation
NMT - Not more than
AAG - Acid Ascorbic 2-Glucoside
AH - Sodium hyaluronate
H₂O - Purified water
DC - Diclofenac sodium
CD - Carvedilol
ECHA - European Chemicals Agency
CIR - Cosmetic Ingredient Review
IARC - International Agency for Research on Cancer
SEM - Standard error of the mean
SD - Standard deviation
CTRL - Control
ANOVA - One-way analysis of variance
TEA - Triethanolamine
DEA - Diethanolamine
INTRODUCTION
1. Introduction

The quick development of new cancer therapeutics/drugs and the growing number of cancer survivors lead to a significant demand to care for these patients. Radiation and/or chemotherapy are required for the several cancers treatment but the use of target therapies has been recurrent. The development of novel targeted anticancer agents, used as monotherapy or in combination with other therapies, improves quality of life (QoL) and cancer patient’s survival rate. [1, 2] The high survival rate has underlined the importance of emotional, social, and medical problems managing as integral mechanisms of continued cancer care. However, the long-term cancer treatments have resulted in several side effects — most notably the cutaneous toxicities (table 1).

Table 1 The most frequent cutaneous toxicities induced by cancer treatments

<table>
<thead>
<tr>
<th>Affected area</th>
<th>Type of toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>Folliculitis</td>
</tr>
<tr>
<td></td>
<td>Rash</td>
</tr>
<tr>
<td></td>
<td>Xerosis</td>
</tr>
<tr>
<td></td>
<td>Keratinocyte proliferation: papilloma</td>
</tr>
<tr>
<td></td>
<td>cyst, keratoacanthoma, Squamous cell skin cancer (cSCC)</td>
</tr>
<tr>
<td></td>
<td>Hand–foot syndrome / Hand-foot skin reaction</td>
</tr>
<tr>
<td></td>
<td>Periorbital edemas</td>
</tr>
<tr>
<td></td>
<td>Photosensitivity</td>
</tr>
<tr>
<td>Nails</td>
<td>Paronychias, pyogenic granulomas</td>
</tr>
<tr>
<td>Hair</td>
<td>Alopecia</td>
</tr>
<tr>
<td>Mucosal</td>
<td>Mucositis, stomatitis</td>
</tr>
<tr>
<td>Cutaneous and hair</td>
<td>Pigmentation disorders</td>
</tr>
</tbody>
</table>

The association of targeted therapies with traditional chemotherapeutic agents led to the increased prevalence of cutaneous toxicities. Hand-foot syndrome (HFS)—also known as palmar-plantar erythrodysesthesia, palmar-plantar erythema, acral erythema, and Burgdorf’s reaction—is one of the most common cutaneous toxicities experienced by patients. The term HFS refers to symptoms related to cytotoxic chemotherapy, whereas the term Hand–foot skin reaction (HFSR) is used when reactions are associated with targeted therapy. [3]
1.1. Clinical findings and Pathogenesis of Hand Foot Syndrome and Hand Foot Syndrome Reaction

Patients under target therapy may experience symptoms as scratchy, burning, or painful sensations in the palms and soles, as well as decreased tolerance to contact (HFSR), throughout the first six weeks of initiating treatment [4]. Subsequent, three clinical phases can be observed: i) symmetrical erythema with painful plaques with typical yellowish discoloration surrounding erythema characterized by an inflammatory phase marked; ii) a hyperkeratotic phase, described as focal hyperkeratosis; and iii) a resolution phase, where the lesions clear quickly after drug cessation or dose decrement (within 1–2 weeks) [5].

In the other hand, regarding HFS symmetrical paresthesia is observed, sideways with desquamative erythema, and edema. These effects tend to be more delayed, appearing with a median time of 72–79 days [6]. Additionally, HFSR affects the soles more frequently than the palms, whereas the palms are more commonly affected in HFS [7].

As already mentioned, HFS is a common cutaneous toxicity correlated to some traditional chemotherapeutic drugs, initially described by Zuehlke in 1974 in association with mitone therapy for the treatment of hypernephroma [8]. In 1980, it was referred to as chemotherapy-induced acral erythema that appeared to be related to cutaneous exposure to chemotherapy but also to targeted drugs (table 2).

Table 2 Summary of the main targeted and chemo therapies that induce HFS

<table>
<thead>
<tr>
<th>Type of toxicity</th>
<th>Causative drugs</th>
<th>Common targets</th>
<th>Patients affected (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hand–foot skin syndrome / Reaction</td>
<td>Vemurafenib, dabrafenib</td>
<td>BRAF</td>
<td>15-20</td>
<td>[9-13]</td>
</tr>
<tr>
<td></td>
<td>Sorafenib, sunitinib, axitinib, pazopanib, regorafenib, vandetanib</td>
<td>VEGFR</td>
<td>10-85</td>
<td>[13-18]</td>
</tr>
<tr>
<td></td>
<td>Docetaxel</td>
<td>DNA</td>
<td>6–37</td>
<td>[19]</td>
</tr>
<tr>
<td></td>
<td>Pegylated liposomal doxorubicin (PLD)</td>
<td>DNA</td>
<td>40–50</td>
<td>[20]</td>
</tr>
<tr>
<td></td>
<td>Doxorubicin</td>
<td>DNA</td>
<td>22-26</td>
<td>[21]</td>
</tr>
<tr>
<td></td>
<td>Docetaxel + capecitabine</td>
<td>DNA</td>
<td>56-63</td>
<td>[22, 23]</td>
</tr>
<tr>
<td></td>
<td>Capecitabine</td>
<td>DNA</td>
<td>50-60</td>
<td>[21, 24]</td>
</tr>
</tbody>
</table>
1.2. Impact of Hand Foot Syndrome on Quality of Life

HFS is not currently considered to be life-threatening, but the location of the lesions can have a significant impact on patients by preventing them from walking or carrying out daily tasks. It has been shown that cutaneous toxicities decrease QoL and impair social functioning in patients receiving oncological therapies. Therefore, the appropriate and effective intervention to manage it can improve QoL [4]. The severity of HFS symptoms depends on the drug dose, peak concentration and total cumulative dose. Consequently, HFS can lead to the cessation of therapy or a dose reduction, and adversely affects QoL and treatment efficacy. The appropriate follow-up of the toxicity is associated with the correct grading of HFS and may also correlate with its impact on QoL. The National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0 (NCI-CTCAE v5.0) is the most commonly used method of grading HFS (table 3). Grade 1 HFS is described as having minimal skin changes or dermatitis with no pain. Grade 2 indicates skin changes with pain and limiting instrumental Activities of Daily Living (ADL). Grade 3, the most severe, is described as skin changes with pain that affect the patient’s ADL.

**Table 3 Hand-Foot Syndrome Common Terminology (NCI-CTCAE v5.0)**

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
<th>Grade 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmar-plantar erythrodynesthesia syndrome</td>
<td>Minimal skin changes or dermatitis (e.g., erythema, edema, or hyperkeratosis) without pain</td>
<td>Skin changes (e.g., peeling, blisters, bleeding, edema, or hyperkeratosis) with pain; limiting instrumental ADL</td>
<td>Severe skin changes (e.g., peeling, blisters, bleeding, edema, or hyperkeratosis) with pain; limiting self care ADL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Basic ADL includes eating, dressing, getting into or out of a bed or chair, taking a bath or shower, and using the toilet [25]. Instrumental activities of daily living are activities associated to independent living like preparing meals, managing money, shopping, doing housework, and/or using a telephone [26, 27]. Nevertheless, other considerations not included in the ADL definition as the ability to clean, do hobbies, work, and exercise are also a very important part of patients’ routine [6].
1.3. **Capecitabine and Hand Foot Syndrome**

As described, many chemotherapy agents can lead to HFS. This cutaneous toxicity is the most common adverse effect of capecitabine, a prodrug (an oral fluoropyrimidine) of 5-fluorouracil (5-FU), completely absorbed in the gastrointestinal tract and favorably activated in tumor tissues.

The capecitabine’s pharmacokinetic profile is characterized by rapid and practically complete gastrointestinal tract absorption. Capecitabine is firstly transformed to 5′-deoxy-5-fluorocytidine (5′-DFCR) by carboxylesterase 2 (CES2), then to 5′-deoxy-5-fluorouridine (5′-DFUR) by cytidine deaminase (CD) in the carcinoma and the liver, and to 5-FU by thymidine phosphorylase (TP), which is presented in neoplastic tissue. [28] Capecitabine is an essential part of chemotherapy for several cancers’ treatment, including colorectal [29], gastric and breast cancer [23] and can imitate the action of continuous 5-FU infusion.

![Figure 1 Metabolic pathways of capecitabine and pharmacologically related targets.](image)

5-10 CH= FH4, 5-10 methylenetetrahydrofolate; 5-10 CH2 FH4, 5-10 methylene-tetrahydrofolate; 5-CH3 FH4, 5-methyltetrahydrofolate; 5-CHO FH4 (FA), 5-formyltetrahydrofolate; DHFR, dihydrofolate reductase; FdUMP, 5-fluorodeoxyuridine 5′-monophosphate; FdUrd, 5-fluorodeoxyuridine; FH2, dihydrofolate; FH4, tetrahydrofolate; MS, methionine synthase; TK, thymidine kinase; TP, thymidine phosphorylase. [30]
The most commonly accepted theory of HFS pathogenesis involves direct toxicity of chemotherapeutic agents against acral epithelium, which leads to an inflammatory process due to overexpression of cyclooxygenase 2 (COX-2; this enzyme catalyzes the conversion of arachidonic acid to prostaglandins) in palms and plantar areas. [28] The inflammatory and mitogenic stimuli that may be triggered directly or indirectly by capecitabine or its metabolites can induce COX 2. [31] Some investigators observed tissues affected by capecitabine and shown several inflammatory changes as white blood cell infiltration, dilated blood vessels, and edema. [32] Therefore, COX-2 inhibitors have been considered as a purpose approach to reduce HFS. The celecoxib effect has been investigated on the occurrence of capecitabine-related HFS in metastatic colorectal cancer. [24, 31, 33] A study with 67 patients with metastatic colorectal cancer found that the addition of celecoxib to capecitabine therapy was reduced the incidence of HFS. Time to tumor progression was 6 months for the capecitabine and celecoxib group versus 3 months for the capecitabine alone group. They also noted that three patients experienced worsening HFS and diarrhea upon discontinuation of celecoxib.[24] Additionally, there are numerous evidences suggesting that the COX-2 overexpression, which can be caused by chemotherapy [34], has a role as an independent prognostic marker for tumor stage, size, and modal status. [35]

**Figure 2** Signaling pathways of inflammation induced by 5-fluorouracil (5-FU). 5-FU activates NFkB which induces expression of pro-inflammatory cytokines (IL-1 β, TNF-α) that promote tissue damage and inflammation (IL-1 β, TNF-α, TGF-β, COX2 and MMP2). (NFkB-the nuclear transcription factor kappa B). Diagram adapted from [34]
Anti-inflammatory agents as celecoxib [a nonsteroidal anti-inflammatory drug (NSAID)] can increase the antitumor activity of capecitabine via inhibition of COX-2 expression.[33] Consequently, COX-2 plays a crucial role in HFS showing that selective COX-2 inhibitors can markedly decrease the incidence of HFS [36].

For HFS in patients undergoing capecitabine therapy, a positive influence of inhibitors of COX-2 has been reported by several studies. [31, 33] However, COX-2 inhibitors only have been investigated as a systemic strategy that can implicate other secondary effects as cardiovascular issues (myocardial infarction, stroke, or heart failure) [24], which impair the risk-benefit ratio.
1.4. **Rational topical drug product design**

Considering the HFS characteristics, its symptoms and the current and available data, it was decided to formulate a drug product containing a NSAID and a beta-adrenergic (βAR) antagonist.

1.4.1. **Topical diclofenac benefit in Hand Foot Syndrome**

Diclofenac, a member of the aryl alkanoic group of nonsteroidal anti-inflammatory drugs (NSAIDs), was synthesized by Ciba-Geigy in the 1960’s and the sodium salt was launched as an oral formulation in 1973. Pharmaceutical compositions of diclofenac for application to the skin have been successfully developed, and a diversity of commercial preparations are currently available. [37]

Diclofenac inhibits COX-2 enzyme with more potency in comparison to COX-1 and is a NSAID with anti-inflammatory, analgesic, and antipyretic properties. [38]

Cordero et al. (1997, 2001) determined a topical efficiency ranking of diclofenac and other NSAIDs based on IC\textsubscript{50} values of COX-2 inhibition and in vitro skin permeation. Diclofenac had the highest value and was proposed by the authors as a good candidate for topical delivery compared with several other NSAIDs studied. [39, 40] Its topical application avoids serious dose-dependent gastrointestinal, cardiovascular and renal adverse effects (figure 3) and represent a valuable option for HFS management.

![Figure 3 Prostaglandin synthesis. Diagram adapted from [41]](image)

Diclofenac has been shown to have a high transdermal penetration and remained a most potent COX-2 inhibitor, and therefore demonstrated its highest anti-inflammatory activity with 50%, 75% and 90% of COX-2 inhibition. [42-44]
1.4.2. Topical Carvedilol benefit in Hand Foot Syndrome

As above mentioned, HFS skin alterations consist of: dilated blood vessels, papillary edema and inflammation.

Carvedilol is a third-generation of non-selective blockers mainly used in the mild to moderate congestive heart failure (CHF) treatment. This drug blocks beta-1 and beta-2 adrenergic receptors (β1, β2AR) as well as the alpha-1 adrenergic receptors (α1AR). Additionally, it is classified according to the Biopharmaceutical Classification System (BCS) as a drug with low solubility (class II) and is presented as an immediate-release oral dosage form in the World Health Organization (WHO) essential drug list [45].

The adrenergic receptors or adrenoceptors are a class of G protein-coupled receptors that are targets of many catecholamines like norepinephrine (NE) and epinephrine (E) but also many drugs like β blockers, β2 agonists and α2 agonists [46]. The respective responsibilities for each adrenoreceptor are described in figure 4.

![Adrenoceptors effects](image)

**Figure 4** Adrenoceptors effects

It is consistent with existing literature that β2ARs are the predominant adrenergic receptor population expressed in cultured keratinocytes. [47] Studies have demonstrated that the release of NE, from the sympathetic terminals in the skin, activates β2ARs on epidermal keratinocytes, conducting to pro-inflammatory mediators as cytokine IL-6 production and secretion (figure 5, [47]). Therefore, carvedilol could inhibit β2Ars and play a significant role on inflammatory decrease.
Considering that vasodilation is a result of circulating adrenaline, this symptom should be abolished by blockade of βAR with carvedilol. Moreover, peripheral vasoconstriction has long been described as a vascular adverse effect of βAR blockers [49] which can explain the premise that carvedilol could conduct to skin vasoconstriction and therefore minimize the HFS symptoms.

Hence, carvedilol presents a promisor role in the reduction of inflammation and vasodilation observed in HFS which are responsible for erythema, edema and pain.

Furthermore, Chang et al. investigated the cancer preventative attributes of carvedilol and the data suggested that carvedilol prevents malignant transformation in vitro and in vivo models of skin carcinogenesis. The results allow concluding that carvedilol may be a safe and new chemopreventive. [50]
1.5. Rational cosmetic product design

As already described, the skin changes are often painful and debilitating and can impact the general activities of daily living and also the QoL. No standard prevention for HFS symptoms has been established yet but taking into account with the characteristic symptoms of HFS (dysesthesia and tingling in the palms, fingers and soles of feet and erythema, which may progress to burning pain with dryness, cracking, desquamation, ulceration and edema), a hypoallergenic product which demonstrates to reinforce skin barrier, could contribute to prevent or minimize some of the referred symptoms and improve the QoL.

In this sense, a benchmarking study on the commercial skin care products was performed and in the absence of any products specially developed for HFS patients (considering its characteristics) or published literature it was considered the products for sensitive skin as the ones which can be applicable in patients with HFS.

Sensitive skin is defined as a sensory reaction activated by contactors and/or environmental factors, typically without an observable clinical manifestation [51]. Although no sign of objective irritation is commonly detected, itching, burning, stinging and a tight sensation are frequently present [52]. Despite the subjective irritation, it is indicated that patients with sensitive skin tend to have less hydrated and flexible and more erythematous and telangiectatic skin, compared to the normal population. Moreover, accentuated differences were found for erythema and hydration/dryness [53].

A compilation of the existing products (on Portuguese market) for sensitive skin was executed by Marta Ferreira, a Master student in the Pharmaceutical Technology Laboratory.

The product search was performed at brand websites and online stores which contained product composition with the following search strategy: TOPIC = (“sensitive skin” OR “sensitive*”). This strategy searched for products that contain the word sensitive and its derivatives in their name. No restrictions were imposed and 228 products (regardless of application zone: body, face or hand) from a total of 31 brands identified as sensitive skin indication.

The present work aimed to evaluate and highlight the relevant ingredients present in this products’ type (table 4) and to assess which ones could be used by patients carrying HSF symptoms and could have a positive impact on the HFS symptoms prevention.
Introduction

Rational design of topical formulations for hand-foot syndrome management

Table 4 Prevalence of relevant ingredients in a total of 228 products for sensitive skin

<table>
<thead>
<tr>
<th>Ingredient – INCI name</th>
<th>Number of cosmetic products</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niacinamide</td>
<td>40</td>
<td>17.54</td>
</tr>
<tr>
<td>Avena sativa</td>
<td>31</td>
<td>13.60</td>
</tr>
<tr>
<td>Allantoin</td>
<td>22</td>
<td>9.65</td>
</tr>
<tr>
<td>Glycyrrhetinic acid (enoxolone)</td>
<td>12</td>
<td>5.26</td>
</tr>
<tr>
<td>Bisabolol</td>
<td>12</td>
<td>5.26</td>
</tr>
<tr>
<td>Aloe barbadensis (aloe vera)</td>
<td>12</td>
<td>5.26</td>
</tr>
<tr>
<td>Laureth-9 (polidocanol)</td>
<td>9</td>
<td>3.95</td>
</tr>
<tr>
<td>Centella asiatica</td>
<td>6</td>
<td>2.63</td>
</tr>
<tr>
<td>Calendula officinalis L.</td>
<td>3</td>
<td>1.32</td>
</tr>
<tr>
<td>Panthenol (Dexpantenol)</td>
<td>2</td>
<td>0.88</td>
</tr>
<tr>
<td>Phytonadione (Vitamin k1)</td>
<td>1</td>
<td>0.44</td>
</tr>
</tbody>
</table>

As a result and considering the biological activity reported on the literature and the rational design presented on this document, enoxolone and calendula were identified as recognized active ingredients for the formulation of a cosmetic product with sensitive skin indication.
1.5.1. Topical enoxolone benefit in Hand Foot Syndrome

Glycyrrhizin is a major saponin of liquorice root (Glycyrrhiza glabra or Licorice), which has been reported to have anti-allergic [54], ocular anti-inflammatory [55] and interferon inducing [56] activities, as well as anti-viral effects [57]. It is a glycone, 18-β-glycyrrhetinic acid (GA; enoxolone), which also has other pharmacological activities as an anti-inflammatory [58] and anti-tumor action [59], in addition to inhibiting the growth of mouse melanoma [60] and the communication across intercellular gap junctions. Moreover, this compound inhibits arachidonic acid-induced mouse ear oedema which is mainly mediated by arachidonic acid metabolites such as leukotriene C4 (LTC4) and prostaglandin E2 (PGE2). [61] Consequently, enoxolone has an interesting achievement profile and may add value to the treatment of inflammatory skin diseases, as HFS.

In cancer treatment by radiation, it has been demonstrated the enoxolone anti-inflammatory activity. Enoxolone suppresses NF-κB activation by inhibiting NF-κB/p65 and IκB-α phosphorylation and decreased the reactive oxygen species (ROS) overproduction in irradiated cells. In an in vivo assay it was demonstrated that this ingredient also alleviated the severity of radiation-induced skin damage, by reducing the inflammatory cell infiltration and cytokines as TNF-α, IL-1β, IL-10 and IL-6 in cutaneous tissues. [62] Additionally, enoxolone provided an anti-inflammatory effect by suppressing the expression and activity of COX-2 enzyme by NF-κB inhibition and phosphoinositide-3-kinase (PI3K) activity. [63]

![Figure 6](image-url) The cyclooxygenase/thromboxane A2 (COX/TxA2) pathway is the pharmacological target of glycyrrhetinic acid (GA)
1.5.2. Topical calendula benefit in Hand foot Syndrome

*Calendula officinalis* belongs to family *Asteracea/Compositae* is native to Central Europe and Mediterranean and there are about 20 species. Calendula grew in European gardens and has been used for medical purposes since 12th century. Mainly, the flowers were prepared into extracts, tinctures, balms, lotions and applied directly to the skin allowing wounds heal and soothing inflamed and damaged skin. The reported pharmacological actions of the plant are: i) angiogenic properties; ii) vascular regeneration activity, affirming the previous activity [64]; iii) analgesic properties were confirmed in the flower extract of the plant, mainly due to triterpenoids which promote anti-inflammatory activity [65]; iv) antimicrobial and antibacterial activity are other pharmacological actions explored up to now [66] and antioxidant and anti-immunomodulatory activities [67]. The hypothesis of having photoprotection effectiveness has also been investigated and positive results were presented. [68]

In this sense, calendula is likely to be effective in HFS prevention because can prevent oxidative stress, has an anti-inflammatory effect and seems to have a safe topical effect in the treatment and prevention of cancer treatment-induced skin-toxicity. [69]

A large phase III randomized trial was conducted by Pommier et al. to compare the prophylactic trolamine with calendula, in preventing acute dermatitis grade 2 or greater in breast cancer patients receiving radiotherapy. This investigation confirmed a markedly decreased acute dermatitis incidence with calendula. [70] Furthermore, other results showed that the anti-inflammatory response of *Calendula officinalis* extract may be mediated by the inhibition of pro-inflammatory cytokines, COX-2, and subsequent prostaglandin synthesis. [65] Braga et al., 2009 confirmed that a propylene glycol extract of *Calendula officinalis* interferes with ROS and reactive nitrogen species (RNS). [71] Additionally, Parente et al., 2012 studied the healing activity of the calendula extract and conclude that *Calendula officinalis* extracts act in a positive way on the inflammatory and proliferative phases of the healing process of skin lesions. [72]

Aside from the referred potential activities, calendula also affects skin architecture. The ingredients derived from calendula improved skin distensibility, direct markers of skin firmness and viscoelasticity, reflecting water content in the epidermis and dermis as demonstrated by Akhtar et al. [73]
1.6. Pre-Formulation and formulation

A gellified emulsion prepared by mixing an emulsion either water-in-oil (W/O) type or O/W with a gelling agent can be named as an emulgel. [74] Its main advantage is the easy incorporation of lipophilic drugs [75] which, due to solubility problems, cannot be formulated directly as hydrogel. For this reason, the emulgel provides better stability and release of the lipophilic drugs in comparison with a hydrogel base.

The main constituents included in an emulgel preparation are water, oils, emulsifiers, gelling agents, and permeation enhancers. [75]

The development of a drug product requires an extensive chemical and physical characterization of the drug substances, active ingredients and excipients, which will triggered a suitable strategy for the development process.

Pre-formulation testing is described as the first step before the rational development of a dosage form. [76] It involves the selection of correct excipients, composition, processing steps and packaging materials and is a learning process before actually developing the dosage forms. The final goal is to design a product this is cost-effective, safe, stable, patient-friendly and therapeutically effective.

Overall, the main objective of pre-formulation testing is to collect sufficient data in to develop a quality, safe and effective product.
Introduction

1.7. Quality target product profile

Described in ICH Q8 guide (R2) published in 2009 as “A prospective summary of the quality characteristics of a drug product […],” the Quality Target Product Profile (QTPP) provides an understanding of what will guarantee the quality, safety, and efficacy of a product for the patient and describes the design criteria for it.

During the initial research steps, the identity, purity or stability of the active substance is established, also pharmaco-toxicology, pharmacokinetics, and clinics are emphasized from the scientific data.

To allow indulging the product to its therapeutic target with the desired concentration, QTPP applies not only the active substance itself but also the dosage form chosen. Therefore, it should be the basis for the development of quality control (QC) attributes (CQAs), control process parameters (CPPs) and control strategy.

As indicated in the ICH Q8 a CQA is considered a property or characteristic physical, chemical, biological or microbiological that must be within a predefined limit/range to confirm the product quality. Besides, the CPP is described as a process parameter whose variability has an impact on a CQA and therefore should be monitored or controlled to ensure the process has desired quality.

Initially, the CQAs should be defined and subsequently, should be acknowledged intermediate CQAs that could affect drug product CQAs. In the end, material attributes (CMAs) and CPPs parameters that may impact the intermediate CQAs of the process should be identified (figure 7).

![Diagram](image)

**Figure 7** Example Approach to Identify Material Attributes and Process Parameters

For the purpose of this work, only CQAs and CPPs were considered through QTPP definition.
AIM
2. Aim of the study

- Rational design of a drug product containing diclofenac sodium and carvedilol for the management of HFS-associated inflammation, burning and erythema caused by capecitabine treatment.
- Rational design of a cosmetic product containing enoxolone and calendula oil with a HFS preventive propose.
- Pre-formulation studies for drug and cosmetic product.
- Definition of a quality target product profile for a drug and cosmetic product.
- Preparation of an emulgel containing diclofenac sodium and carvedilol and an emulgel containing enoxolone and calendula oil.
- Formulations' optimization.
- Characterization of the prepared formulations.
EXPERIMENTAL PART
3. Experimental part

3.1. Material and Methods

3.1.1. Pre-formulation study

The biological and chemical characteristics as well as hazard potential, stability and incompatibilities of the actives and excipients to be used in the formulations were studied considering the literature research. The carvedilol solubility in calendula oil and propylene glycol was determined in duplicate samples. It was added to 0.1 g of carvedilol quantities of calendula oil or propylene glycol progressively larger (0.1 ml, 0.5 ml, 1 ml, 2 ml, 10 ml, 30ml and 50 ml) until 50 ml into a graduated cylinder. The origin of the raw materials used in this work is listed in annex I.

3.1.2. Bioactivity tests

The assessment of inducible nitric oxide synthase (NOS) mediated nitric oxide (NO) accumulation and cellular viability test were performed under the supervision of Prof. Dr. Maria Teresa Cruz Rosete from the Faculty of Pharmacy and Centre for Neuroscience and Cell Biology of University of Coimbra. The cell culture and cell handling were performed by Prof. Dr. Maria Teresa Cruz Rosete and some microplate readings were performed with the collaboration of the researchers Isabel Ferreira and Ana Silva.

3.1.2.1. Assessment of inducible nitric oxide synthase mediated NO accumulation

RAW 264.7, a mouse leukemic macrophage cell line from the American Type Culture Collection (ATCC TIB-71) was cultured on endotoxin-free Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10% (v/v) non-inactivated fetal bovine serum, 3.02 g/L sodium bicarbonate, 100 µg/mL streptomycin and 100U/mL penicillin at 37°C in a humidified atmosphere of 95% air and 5% CO₂ (carbon dioxide). During the experiments, cells were monitored through microscope observation to detect any morphological change. Experiments were carried out at least three times.
Materials and Methods

To perform the anti-inflammatory assays, RAW 264.7 cells were suspended in culture medium and adjusted to 60,000 cells per well in a final volume of 200 µl. Then, the cells were incubated with several concentrations of the chemicals (diclofenac sodium, enoxolone and calendula oil) in the absence or the presence of LPS (Lipopolysaccharides; 50 ng/ml) for 24 h at 37°C. Thereafter, culture supernatants were removed and accumulated NO, as a measure of NOS activity, was assessed in cell-free culture supernatants by the Griess reaction.

This method is based on a diazotization reaction and was firstly described by Griess in 1879. The Griess reagent contains 1% sulfanilamide and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride (NED) in acid conditions (Phosphoric acid; H₃PO₄, 5% v/v) to quantify the major metabolites of NO such as nitrite and nitrate, in a variety of biological fluids, such as plasma, serum, urine and culture medium. Formed nitrite (NO₂⁻) in medium reacts under acidic conditions with sulfanilamide to form a diazonium cation which subsequently couples to the aromatic amine NED to produce red-violet, water-soluble azo dye. 100 µl of culture supernatant was then mixed with an equal volume of Griess reagent (1% sulphanilamide and 0.1% naphthylethlenediamine in 5% phosphoric acid) and after 30 min of incubation, color development was assessed at 550 nm with a microplate reader (Biotek Synergy HT (BioTek Instruments, Winooski, VT, USA).

The tests were performed in a concentration range of diclofenac sodium (1 µM and 70 µM) and enoxolone (4 µM and 40 µM). For Calendula oil, a range of volumes was used 3.2 to 29.4 µL (16 to 147 µL/mL).

Stock solutions (10 mM) of diclofenac sodium and enoxolone were prepared in dimethyl sulfoxide (DMSO) and stored at room temperature and protected from the light until used. Serial dilutions (20x) of tested solutions in culture medium were prepared. DMSO concentrations ranged from 0.04% to 0.7% (v/v).

This study evaluates the effect of diclofenac sodium, enoxolone and calendula on NO production evoked by LPS in macrophages. As a positive control, we used cells exposed to LPS where it is expected the highest NO production for this condition. Meanwhile, the negative control of the study consists of untreated cells, for which it is expected a low NO production.

Additionally, a DMSO control was used to ensure that the observed anti-inflammatory effect is not due to the presence of DMSO and cell viability is not compromised since DMSO concentrations higher than 0.2% could present some cytotoxicity and also anti-inflammatory activity.
3.1.2.2. **Cellular viability test (AlamarBlue®)**

In this experiment, macrophages were treated with different concentrations of diclofenac, enoxolone and calendula oil in duplicates for 24 h. Cell cultures were incubated at 37ºC and 5% CO₂. After incubation, the compounds were gently aspirated to avoid interference with the proliferation assay due to physical cell damage. 100µL of resazurin solution (final in good concentration of 50 µM) were added and cell cultures were further incubated for 3h at 37ºC protected from light. As only viable cells can reduce resazurin (a non-fluorescent blue dye) into resorufin (pink and fluorescent), their number correlates with the magnitude of dye reduction. Quantification of resorufin was performed on a Biotek Synergy HT (BioTek Instruments, Winooski, VT, USA) plate reader at 570 nm, with a reference wavelength of 620 nm. Experiments were carried out at least in three independent experiments.

- **Statistical analysis**

Bioassays were measured in duplicate and three different and independent experiments. Results were expressed as mean ± SEM. One-way analysis of variance (ANOVA) followed by an independent samples t-test which compares the means for two groups was applied to calendula oil results (since only for calendula oil three complete independent experiments were observed). Statistical differences were calculated and represented with the following symbols of significance level ** p < 0.01, *** p < 0.001 and # p < 0.05. Statistical analysis was performed using Excel® software.
3.1.3. Quality target product profile definition

3.1.3.1. Quality Target Product Profile for drug product

The pre-defined CQAs are present in the following table. On this work, only the physical attributes were considered with the exception of particle distribution size (PSD) was not studied. Regarding the stability point, preliminary physical stability was performed.

Table 5 Quality Target product profile (QTPP) for the drug product formulation

<table>
<thead>
<tr>
<th>QTPP</th>
<th>Target</th>
<th>Justification</th>
<th>CQAs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dosage form</td>
<td>Emulgel</td>
<td>Helps providing the localized therapy for reduce the HFS symptoms.</td>
<td></td>
</tr>
<tr>
<td>Route of</td>
<td>Topical</td>
<td>The proposal rout of delivery is external application with low residue and easy spreadability.</td>
<td></td>
</tr>
<tr>
<td>administration</td>
<td></td>
<td>All the proposed values are within the effective concentrations required for localized effects as per literature.</td>
<td></td>
</tr>
<tr>
<td>Dosage strength</td>
<td>2 % w/w diclofenac</td>
<td>Emulsion gelled with a gelling agent containing diclofenac and carvedilol.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5 % w/w carvedilol</td>
<td>This formulation type can potentially contribute to improve the patient adherence.</td>
<td></td>
</tr>
<tr>
<td>Dosage form</td>
<td>Emulgel</td>
<td>Physical appearance is not considered critical as it is not directly linked to safety and efficacy.</td>
<td></td>
</tr>
<tr>
<td>design</td>
<td></td>
<td>Is considered critical as it is directly linked to safety and efficacy.</td>
<td>Yes</td>
</tr>
<tr>
<td>Appearance</td>
<td></td>
<td>Is considered critical as it is directly linked to safety and efficacy.</td>
<td>Yes</td>
</tr>
<tr>
<td>Identification</td>
<td>Positive for diclofenac and carvedilol.</td>
<td>Is considered critical as it is directly linked to safety and efficacy.</td>
<td>Yes</td>
</tr>
<tr>
<td>Assay (QC specification)</td>
<td>90-110%</td>
<td>Is considered critical as it is directly linked to safety and efficacy.</td>
<td>Yes</td>
</tr>
<tr>
<td>Impurities /</td>
<td>Meet Ph. Eu.</td>
<td>Is considered critical as it is directly linked to safety and efficacy.</td>
<td>Yes</td>
</tr>
</tbody>
</table>
## Materials and Methods

### Rational design of topical formulations for hand-foot syndrome management

<table>
<thead>
<tr>
<th>QTPP</th>
<th>Target</th>
<th>Justification</th>
<th>CQAs</th>
</tr>
</thead>
<tbody>
<tr>
<td>degraded products</td>
<td>Top, middle and bottom</td>
<td>directly linked to safety and efficacy.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Individual results 90-110%</td>
<td>Is considered critical as it is directly linked to safety and efficacy.</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>105.0%, and RSD NMT 5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homogeneity</td>
<td>Spreadability</td>
<td>Is considered critical as it is directly linked to safety and efficacy.</td>
<td>Yes</td>
</tr>
<tr>
<td>(QC specification)</td>
<td>Evaporation rate and residue PSD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical attributes</td>
<td>Methyl Paraben: 0.05% to 0.25%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Propyl Paraben: 0.02% to 0.04%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preservatives content</td>
<td>Meet Ph. Eu.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(QC specification)</td>
<td>No failure.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microbiological</td>
<td>Physical stability (No less than 12-months</td>
<td>To maintain the optimum strength of API for therapeutic efficacy during the storage period.</td>
<td>Yes</td>
</tr>
<tr>
<td>(QC specification)</td>
<td>expiration dating period)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Package integrity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stability</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CPP can be defined as the time and type of mixture and the temperature used during the process. These parameters were defined and assess through the formulation process.
3.1.3.2. Quality Target Product Profile for cosmetic product

The pre-defined CQAs are present in the following table. On this work, only the physical attributes and preliminary physical stability were considered.

Table 6 Quality Target product profile for a cosmetic product formulation

<table>
<thead>
<tr>
<th>QTPP</th>
<th>Target</th>
<th>Justification</th>
<th>CQAs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dosage form</td>
<td>Emulgel</td>
<td>Helps preventing the HFS symptoms.</td>
<td></td>
</tr>
<tr>
<td>Route of administration</td>
<td>Topical</td>
<td>The proposal rout of delivery is external application with low residue and easy spreadability.</td>
<td></td>
</tr>
<tr>
<td>Dosage strength</td>
<td>1 % w/w enoxolone</td>
<td>These strengths are within the effective concentrations required for localized effects as per literature.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.5 % w/w calendula oil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dosage design</td>
<td>Emulsion gelled with a gelling agent containing enoxolone and calendula oil.</td>
<td>This formulation type is intend to improve the patient adhesion.</td>
<td></td>
</tr>
<tr>
<td>Appearance</td>
<td>Yellow to off-yellow, uniform and homogeneous.</td>
<td>Physical appearance is not considered critical as it is not directly linked to safety and efficacy.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No unpleasant odor.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Identification</td>
<td>Positive for enoxolone and calendula.</td>
<td>Is considered critical as it is directly linked to safety and efficacy. Yes</td>
<td></td>
</tr>
<tr>
<td>Assay (QC specification)</td>
<td>90-110%</td>
<td>Is considered critical as it is directly linked to safety and efficacy. Yes</td>
<td></td>
</tr>
<tr>
<td>Impurities / degraded products</td>
<td>Meet Ph. Eu.</td>
<td>Is considered critical as it is directly linked to safety and efficacy. Yes</td>
<td></td>
</tr>
<tr>
<td>Homogeneity (QC specification)</td>
<td>Top, middle and bottom Individual results 90-110</td>
<td>Is considered critical as it is directly linked to safety and efficacy. Yes</td>
<td></td>
</tr>
</tbody>
</table>
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Rational design of topical formulations for hand-foot syndrome management

<table>
<thead>
<tr>
<th>QTPP</th>
<th>Target</th>
<th>Justification</th>
<th>CQAs</th>
</tr>
</thead>
<tbody>
<tr>
<td>%, average 95-0-105.0%, and RSD NMT 5%</td>
<td>efficacy.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical attributes</td>
<td>Spreadability Evaporation rate and residue.</td>
<td>Is considered critical as it is directly linked to safety and efficacy and patient adhesion.</td>
<td>Yes</td>
</tr>
<tr>
<td>Preservatives content (QC specification)</td>
<td>Methyl Paraben: 0.05% to 0.25% Propyl Paraben: 0.02% to 0.04%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microbiological (QC specification)</td>
<td>Meet Ph. Eur.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Package integrity</td>
<td>No failure Physical stability (No less than 12-months expiration dating period).</td>
<td>To maintain the optimum strength of active ingredients.</td>
<td>Yes</td>
</tr>
</tbody>
</table>

CPP can be defined as the time and type of mixture and the temperature used during the process. These parameters were defined and assessed through the formulation process.
3.1.4. Formulation

All formulations were identified according to a pre-defined nomenclature present in annex II.

3.1.4.1. Topical base preparation

The composition of the three tested bases for emulgel formulation is listed in the following table 7.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Function / Biological activity</th>
<th>Formulations tested</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F1</td>
</tr>
<tr>
<td>Almond oil</td>
<td>Emollient</td>
<td>9%</td>
</tr>
<tr>
<td>Calendula oil</td>
<td>Anti-inflammatory agent</td>
<td>-</td>
</tr>
<tr>
<td>Tween® 80</td>
<td>Emulsifying agent</td>
<td>1%</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>Humectant</td>
<td>-</td>
</tr>
<tr>
<td>Glycerin</td>
<td>Humectant</td>
<td>7.5%</td>
</tr>
<tr>
<td>Sodium Hyaluronate (AH)</td>
<td>Humectant/Surfactant</td>
<td>1%</td>
</tr>
<tr>
<td>Carbopol® 980</td>
<td>Gelling agent</td>
<td>0.7%</td>
</tr>
<tr>
<td>Paraben stock solution 4)</td>
<td>Preservative</td>
<td>2.1 ml</td>
</tr>
<tr>
<td>Triethanolamine (TEA)</td>
<td>pH balancer</td>
<td>qs pH 6</td>
</tr>
<tr>
<td>Purified water</td>
<td></td>
<td>qs 30 g</td>
</tr>
</tbody>
</table>

At first, carbopol® 980 was joined with one part of purified water (approximately 10.4 g) and mixed for 2 hours with magnetic stirring. At the same time, sodium hyaluronate (AH), glycerin (F1 and F2) or propylene glycol (F3) and preservative were joined with another part of water and mixed for 2 hours with magnetic stirring (AH was added at intermittently). When both mixtures were homogeneous they were mixed and drops of triethanolamine (TEA) were added (3 drops) until pH=6 (test strip). The mixture was transferred to a Microcaya Unguator container and the almond and/or calendula oil and tween® 80 were joined and stirred for 2 min. (speed position was set at II in the XIII option range).

4) The paraben stock solution was prepared with 7 g of methyl paraben and 97.4 g of propylene glycol.
The preparation method was similar for F1, F2 and F3 formulations. However, some quantities and ingredients adjustments were made. In F2 formulation, unintentionally, it was used 9% of tween® 80 instead of 1% but since the formulation showed similar characteristics and this excipient stabilizes the preparation, it was proposed to use 5% of tween® 80 in F3 formulation. Additionally, considering carbopol® 980 it was decided to increase its concentration to 0.15% in F3 formulation in order to improve the rheological properties. Glycerin was substituted by propylene glycol to achieve a better dissolution of the drug substances that will be used in drug product and the active ingredients in the cosmetic product.
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3.1.4.2. Topical drug product preparation

The composition of the first three tested drug product is listed in the following table 8.

Table 8 Composition of the drug product formulations

<table>
<thead>
<tr>
<th>Compound</th>
<th>Formulations tested</th>
<th>F3 A\textsubscript{DC/CD}, F3 B\textsubscript{DC/CD+AAG}, F3 C\textsubscript{DC/CD+AAG}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diclofenac sodium</td>
<td>2%</td>
<td></td>
</tr>
<tr>
<td>Carvedilol</td>
<td>0.5%</td>
<td></td>
</tr>
<tr>
<td>Almond oil</td>
<td>4.5%</td>
<td></td>
</tr>
<tr>
<td>Calendula oil</td>
<td>4.5%</td>
<td></td>
</tr>
<tr>
<td>Tween\textsuperscript{®} 80</td>
<td>5%</td>
<td></td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>7.5%</td>
<td></td>
</tr>
<tr>
<td>Sodium hyaluronate (AH)</td>
<td>1%</td>
<td></td>
</tr>
<tr>
<td>Carbopol\textsuperscript{®} 980</td>
<td>0.85%</td>
<td></td>
</tr>
<tr>
<td>Paraben stock solution</td>
<td>2.1 ml</td>
<td></td>
</tr>
<tr>
<td>Acid Ascorbic 2-Glucoside (AAG)</td>
<td>1% - F3 B\textsubscript{DC/CD+AAG}</td>
<td></td>
</tr>
<tr>
<td>Triethanolamine</td>
<td>qs pH 6</td>
<td></td>
</tr>
<tr>
<td>Purified water</td>
<td>q.b. 30g</td>
<td></td>
</tr>
</tbody>
</table>

Carvedilol was dissolved in propylene glycol, at a temperature of 30\textdegree C, during 1h30min, with magnetic agitation (Mixture A).

Carbopol\textsuperscript{®} 980 was joined with one part of purified water and mixed for 2 hours with a magnetic stirrer at room temperature (Mixture B).

From this step on, three different formulations (F3 A\textsubscript{DC/CD}, F3 B\textsubscript{DC/CD+AAG}, F3 C\textsubscript{DC/CD+AAG}) were prepared, considering the incorporation of diclofenac into the aqueous phase.

For F3 A\textsubscript{DC/CD}, which did not contain AAG, diclofenac sodium was joined to AH and a part of purified water and mixed for 2 hours with a magnetic stirrer at room temperature (Mixture C).

In F3 B\textsubscript{DC/CD+AAG}, diclofenac sodium was added to a part of purified water and mixed for 1h30min., with a magnetic stirrer at room temperature. Then, AH was added to the previous dispersion and mixed for 15min. at room temperature with a magnetic stirrer.
(Mixture C). The AAG was added to mixture C obtaining mixture D.
For F3 $\text{DC/CD+AG}$, diclofenac was added to a part of purified water mixed for 1h30min. at room temperature and then more 30 min. at 50ºC with a magnetic stirrer. The AH was added to the previous dispersion and mixed for 15min. at room temperature with a magnetic stirrer (Mixture C). The AAG was added to mixture C obtaining mixture D.

Hereafter and common to the three formulations, the mixture A was added to mixture C or D, at room temperature, during 15 min., with magnetic agitation. Then, the mixture B and preservative were blended with magnetic agitation. The obtained mixture was transferred to a Microcaya Unguator container and the almond oil, calendula oil and tween® 80 were joined and stirred for 2 min. (speed position was set at II in the XIII option range).

These three formulations were not homogenous and too many difficulties in the carvedilol and diclofenac solubilization were faced leading to the decision to remove the drug substance carvedilol from the formulation.

In this sense, a new formulation (with the same component's concentration of F3C $\text{DC/CD+AAG}$) only with diclofenac sodium as drug substance was prepared (F3 $\text{DC+AAG}$).

For F3 $\text{DC+AAG}$ formulation, diclofenac was dissolved in propylene glycol and a part of purified water, at a temperature of 30ºC, during 30min., with magnetic agitation (Mixture A).
AH was added to another part of purified water mixed for 30 min. with a magnetic stirrer at 30ºC and then AAG was joined and mixed for more 20 min. in the same conditions (Mixture B).
Carbopol® 980 was joined with the third part of purified water and mixed for 1h30min. with a magnetic stirrer at room temperature (Mixture C).
Mixtures A and B were joined and mixed for 30 min. with a magnetic stirrer at room temperature, forming mixture D.
Mixture D was joined to mixture C and mixed for 15 min. with a magnetic stirrer at room temperature. The preservative was added and mixed for more 5 min. at the same conditions (Mixture E).
The obtained mixture was transferred to a Microcaya Unguator container and the oils and tween® 80 were joined and stirred for 4 min. (speed position was set at II in the XIII option range; figure 8).
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Figure 8 F3 DC+AAG preparation method (AH - Sodium Hyaluronate, AAG - Acid Ascorbic 2-Glucoside, H₂O – purified water).

The use of TEA was not necessary for pH balance.

In this formulation (F3 DC+AAG) several agglomerates were observed and the addition of AAG to AH led to a sticky gel formation. The AAG was added to the formulation due to its antioxidant properties, however, and in line with the described phenomena, it was necessary to remove AAG in order to optimize the method preparation.

Consequently, it proceeds with the preparation of an emulgel, described as MED, with the following composition (table 9).

Table 9 Composition of the optimized drug product formulation

<table>
<thead>
<tr>
<th>Compound</th>
<th>MED Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diclofenac sodium</td>
<td>2%</td>
</tr>
<tr>
<td>Almond oil</td>
<td>4.5%</td>
</tr>
<tr>
<td>Calendula oil</td>
<td>4.5%</td>
</tr>
<tr>
<td>Tween® 80</td>
<td>5%</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>7.5%</td>
</tr>
<tr>
<td>Sodium hyaluronate (AH)</td>
<td>1%</td>
</tr>
<tr>
<td>Carbopol® 980</td>
<td>0.85%</td>
</tr>
<tr>
<td>Paraben stock solution</td>
<td>2.1 ml</td>
</tr>
<tr>
<td>Purified water</td>
<td>q.b. 30g</td>
</tr>
</tbody>
</table>
3.1.4.3. Topical cosmetic product preparation

The cosmetic product composition is the same as the optimized drug product formulation but with enoxolone as the active ingredient and without diclofenac sodium. Its preparation is described below.

\[\text{Enoxolone} + \text{H}_2\text{O} + \text{propylene glycol} \]

\[\text{AH} + \text{H}_2\text{O} \]

\[\text{Carbopol} + \text{H}_2\text{O} \]

\[\text{magnetic agitation during 1h30min at room temperature} \]

\[\text{magnetic agitation during 1h30min at room temperature} \]

\[\text{magnetic agitation during 5min at room temperature} \]

\[+ \text{Paraben stock solution} \]

\[+ \text{Oils + tween}^\circledast 80 \]

\[\text{Ungator 5min} \]

\[\text{Ungator 5min} \]

\[\text{COSM} \]

**Figure 9.** Cosmetic preparation method (AH - Sodium Hyaluronate; H\(_2\)O – purified water)

Enoxolone was dissolved in propylene glycol and purified water during 1h30min., with magnetic agitation at room temperature (Mixture A).

AH was joined with one part of water and mixed for 1h30, with a magnetic stirrer, at room temperature (Mixture B). Carbopol\(^\circledast\) 980 was joined with one part of purified water and mixed for 1h30min. in a magnetic stirrer, at room temperature (Mixture C).

Mixture B was added to mixture C at room temperature, during 5 min., with magnetic agitation and it was joined the preservative with magnetic agitation. The mixture was transferred to the container of a mechanical homogenizer (Microcaya Unguator) and the oils and tween\(^\circledast\) 80 were joined and stirred for 5 min. (speed position was set at II in the XIII option range).

Finally, the mixture A was added to mixture F and blended for 5 min. in the Unguator (speed position was set at II in the XIII option range).
3.1.5. Characterization of the topical formulations

3.1.5.1. Organoleptic characteristics
Organoleptic assessment, particular features of the prepared emulgels, such as appearance, homogeneity, odor and color were evaluated using the scale of 0 (bad), + (moderate), ++ (good), +++ (best). [77]

3.1.5.2. Spreadability
The textural analysis was performed in the compression mode in a texturometer (TAXT2i Texture Analyser®) by carrying out a spreadability test (cone and matched female probes). Measurements were performed in triplicate at 20°C (in the same conditions), using the emulgel. The parameter negative area (correlated with adhesiveness) was calculated from the texturogram. The maximum force correlates with the firmness of the formulations. [78]

3.1.5.3. pH
As these formulations, will be applied to the skin (pH around 5-5.5), it is important to ensure skin compatibility and stability. For this test, it was used a pH strip to measure the emulgel pH, at room temperature (23°C) for base formulations and a pH meter [Medidor PH BASIC 20, Crison, Spain] for drug and cosmetic formulations.

3.1.5.4. Evaporation rate and residue
Evaporation rate and residue were evaluated after the application of the emulgel (1.5 mg/cm²) in polymethyl methacrylate plates (supplied by Schönberg GmbH, Hamburg and with an area of 2 cm² and standard roughness of 5 μm) and weighing of the plates at different times, using the analytical balance RADWAG AS 220.r2, during 60 min. The results were compared with petrolatum since it forms a water-repellent film around the applied area, creating an effective barrier against the evaporation of the skin’s natural moisture.

3.1.5.5. Preliminary physical stability
To evaluate the possibility of going forward with stability study of the emulgel, centrifugation with Eppendorf Centrifuge, using 3000 rpm, for 30 min. was performed. The emulgel was balanced with distilled water.
3.2. Results and discussion

3.2.1. Pre formulation study

This section presents some biological and chemical characteristics as well as hazard potential, stability and incompatibilities of the pre-selected actives and excipients to be used in the formulations considering the literature research (tables 10 and 11).

**Table 10 Active ingredients and drug substances.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Biological activity</th>
<th>Log P</th>
<th>pH</th>
<th>Solubility</th>
<th>Hazard potential</th>
<th>Incompatibilities</th>
<th>Stability</th>
<th>Ref. and databases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium diclofenac</td>
<td>Inhibitor COX-2</td>
<td>4.75</td>
<td></td>
<td>Soluble in ethanol</td>
<td>No</td>
<td>Hypotensive Agents, Calcium Channel Blockers, Insulin or Oral Hypoglycemics</td>
<td>Stable under ordinary conditions but degradation was showed at higher than 65°C.</td>
<td>[79] PubChem</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Poor solubility in water (50mg/L)</td>
<td></td>
<td></td>
<td>Free solubility in methylene chloride, methanol and propylene glycol.</td>
<td>[80]</td>
</tr>
<tr>
<td>Carvedilol</td>
<td>β-blocker</td>
<td>4.19</td>
<td></td>
<td>Practically insoluble in water (0.583 mg/L)</td>
<td>No</td>
<td>CYP2D6 Inhibitors, Hypotensive Agents, Cyclosporine Inducers/Inhibitors of Hepatic Metabolism, Calcium Channel Blockers, Insulin or Oral Hypoglycemics</td>
<td>Degradation was not observed when subjected to stress conditions like acid hydrolysis. Carvedilol was degraded to impurities A, B, C, D, E &amp; unknown</td>
<td>[81] PubChem</td>
</tr>
</tbody>
</table>
## Results and Discussion

### Rational design of topical formulations for hand-foot syndrome management

<table>
<thead>
<tr>
<th>Compound</th>
<th>Biological activity</th>
<th>Log P</th>
<th>pH</th>
<th>Solubility</th>
<th>Hazard potential</th>
<th>Incompatibilities</th>
<th>Stability</th>
<th>Ref. and databases</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Enoxolone</strong></td>
<td>Antiallergic, antibacterial, and antiviral properties.</td>
<td>6.4</td>
<td>4.0-5.0</td>
<td>Insoluble in water</td>
<td>No</td>
<td>Oxidizing agents</td>
<td>Stable under ordinary conditions.</td>
<td>Pubchem echa.europa.eu</td>
</tr>
<tr>
<td></td>
<td>It is used topically (for allergic or infectious skin inflammation and orally).</td>
<td></td>
<td></td>
<td>Freely soluble in pyridine, chloroform and dioxane.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Antibacterial, Anti-inflammatory, Antioxidant, Antiproliferative, etc.</td>
<td></td>
<td></td>
<td>Soluble in ethanol and Propylene glycol. Insoluble in petroleum ether.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Calendula oil</strong></td>
<td></td>
<td>8.3</td>
<td>4.0-5.0</td>
<td>Oil-soluble</td>
<td>No</td>
<td>Some sedative medications</td>
<td>Stable under ordinary conditions.</td>
<td>Pubchem</td>
</tr>
</tbody>
</table>
## Results and discussion

### Rational design of topical formulations for hand-foot syndrome management

Table 11 Excipients.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Functional category</th>
<th>Log P</th>
<th>pH</th>
<th>Solubility</th>
<th>Hazard potential</th>
<th>Incompatibilities</th>
<th>Stability</th>
<th>Ref. and databases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Almond oil</td>
<td>Emollient; oleaginous vehicle; solvent</td>
<td>3.3</td>
<td>4 - 5</td>
<td>Miscible with chloroform, and ether. Slightly soluble in ethanol (95%).</td>
<td>No</td>
<td>no information</td>
<td>Should be stored in a well-closed container in a cool, dry place away from direct sunlight and odors. Does not easily turn rancid.</td>
<td></td>
</tr>
<tr>
<td>Tween® 80</td>
<td>Emulsifying agent; nonionic surfactant; solubilizing agent; wetting, dispersing/suspending agent.</td>
<td>2.39</td>
<td>6 - 8</td>
<td>Very soluble in water; soluble in alcohol, cottonseed oil, corn oil, ethyl acetate, methanol, toluene; insoluble in mineral oil.</td>
<td>Moderately toxic by IV route. Mildly toxic by ingestion Eye irritation Experimental tumorigen, reproductive effects Mutagenic data.</td>
<td>Discoloration and/or precipitation occur with various substances, especially phenols, tannins, tarls, and tarlike materials. The antimicrobial activity of paraben preservatives is reduced in the presence of polysorbates.</td>
<td>Polysorbates are stable to electrolytes and weak acids and bases; gradual saponification occurs with strong acids and bases. The oleic acid esters are sensitive to oxidation. Polysorbates are hygroscopic and should be examined for water content prior to use and dried if necessary. Also, in common with other polyoxyethylene surfactants, prolonged storage can lead to the formation of peroxides.</td>
<td></td>
</tr>
</tbody>
</table>

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### Results and discussion

#### Rational design of topical formulations for hand-foot syndrome management

<table>
<thead>
<tr>
<th>Compound</th>
<th>Functional category</th>
<th>Log P</th>
<th>pH</th>
<th>Solubility</th>
<th>Hazard potential</th>
<th>Incompatibilities</th>
<th>Stability</th>
<th>Ref. and databases</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Propylene glycol</strong></td>
<td>Antimicrobial preservative; disinfectant; humectant; plasticizer; solvent; stabilizer for vitamins; water-miscible cosolvent</td>
<td>-0.79</td>
<td>6-8</td>
<td>Miscible with acetone, chloroform, ethanol (95%), glycerin, and water. Soluble at 1 in 6 parts of ether. Not miscible with light mineral oil or fixed oils, but will dissolve some essential oils.</td>
<td>In topical preparations, propylene glycol is regarded as minimally irritant, although it is more irritant than glycerin. Some local irritation is produced upon application to mucous membranes or when it is used under occlusive conditions. Parenteral administration may cause pain or irritation when used in high concentration.</td>
<td>Propylene glycol is incompatible with oxidizing reagents such as potassium permanganate.</td>
<td>Stable in a well-closed container, but at high temperatures, in the open, it tends to oxidize.</td>
<td>[83] PubChem ChemicalBook</td>
</tr>
<tr>
<td><strong>Sodium hyaluronate</strong></td>
<td>Humectant; lubricant; matrix for sustained release.</td>
<td>-6.623</td>
<td>5.0-8.5</td>
<td>Soluble in water, although speed of dissolution depends upon molecular weight (higher molecular weights are slower to dissolve, although this process can be increased by gentle agitation). Slightly soluble in mixtures of organic solvents with water.</td>
<td>No</td>
<td>Incompatible with strong oxidizing agents. Between 37°C and 60 ºC, only moderate degradation was observed for hyaluronic acid solutions.</td>
<td>No</td>
<td>[84] ChemicalBook</td>
</tr>
</tbody>
</table>
## Results and discussion

### Rational design of topical formulations for hand-foot syndrome management

<table>
<thead>
<tr>
<th>Compound</th>
<th>Functional category</th>
<th>Log P</th>
<th>pH</th>
<th>Solubility</th>
<th>Hazard potential</th>
<th>Incompatibilities</th>
<th>Stability</th>
<th>Ref. and databases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbopol® 980</td>
<td>Bioadhesive; emulsifying agent; release-modifying agent; suspending agent; tablet binder; viscosity-increasing agent</td>
<td>0.257</td>
<td>2.5 - 3</td>
<td>Soluble in water and, after neutralization, in ethanol (95%) and glycerin.</td>
<td>No</td>
<td>Caromers are discolored by resorcinol and are incompatible with phenol, cationic polymers, strong acids, and high levels of electrolytes. Certain antimicrobial adjuvants should also be avoided or used at low levels. Trace levels of iron and other transition metals can catalytically degrade caromer dispersions. Intense heat may be generated if a caromer is in contact with a strong basic material such as ammonia, potassium or sodium hydroxide, or strongly basic amines. Certain amino-functional actives form water-insoluble complexes with caromer; often this can be prevented by adjusting the solubility parameter of the fluid phase using appropriate alcohols and polyols. Caromers also form pH-dependent complexes with certain polymeric excipients. Adjustment of solubility parameter can also work in this situation.</td>
<td>No decomposition during appropriate use.</td>
<td>Pubchem</td>
</tr>
</tbody>
</table>
## Results and discussion

### Rational design of topical formulations for hand-foot syndrome management

<table>
<thead>
<tr>
<th>Compound</th>
<th>Functional category</th>
<th>Log P</th>
<th>pH</th>
<th>Solubility</th>
<th>Hazard potential</th>
<th>Incompatibilities</th>
<th>Stability</th>
<th>Ref. and databases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid Ascorbic 2-Glucoside</td>
<td>Antioxidant</td>
<td>-3.1</td>
<td>2.3-2.4</td>
<td>Soluble in water (879 g/L) at 25°C.</td>
<td>Not expected to be a health hazard when used under normal conditions.</td>
<td>Keep away from light and moisture, to be stored in cool and dry place at room temperature.</td>
<td>Highest stability was determined at 55.3 ºC and pH 6.4</td>
<td>Pubchem</td>
</tr>
<tr>
<td>Triethanolamine</td>
<td>pH balancer</td>
<td>-1.59</td>
<td>12.2</td>
<td>Miscible in water insoluble in benzene, ether, and petroleum distillates, miscible with methanol or acetone; sparingly soluble in hydrocarbon solvents; readily forms salts with organic and inorganic acids.</td>
<td>Clinical skin testing of TEA and cosmetic products containing TEA and DEA showed mild skin irritation in concentrations above 5%.</td>
<td>Incompatible with metals such as aluminum and copper, halogenated organics, strong acids, oxidizing materials and absorbent materials.</td>
<td>Stable under ordinary conditions.</td>
<td>CIR</td>
</tr>
</tbody>
</table>
3.2.1.1. Carvedilol solubility

The carvedilol solubility in calendula oil and propylene glycol was determined in duplicate samples. In the case of calendula oil even with the addition of 50 ml to 0.1 g of powder, the carvedilol was not dissolved (figure 10).

![Carvedilol solubility test in calendula oil](image1)

**Figure 10** Carvedilol solubility test in calendula oil

In the case of propylene glycol, it was necessary to add up to 50 ml to 0.1 g of powdered carvedilol to achieve complete solubilization (figure 11). Carvedilol is therefore considered sparingly soluble in propylene glycol. Therefore, the glycerin was replaced by propylene glycol.

![Carvedilol solubility test in propylene glycol](image2)

**Figure 11** Carvedilol solubility test in propylene glycol
Results and discussion

Rational design of topical formulations for hand-foot syndrome management

3.2.2. Topical base formulations

The common semisolid formulations for cutaneous application include ointments, pastes, gels and creams. However, for skin care, the most used and appreciated formulations are creams and emulgels. [86] One of the most complicated problems related to the formulation and production of these preparations is the establishment of reliable techniques for their characterization, mainly due to the complexity of their physical structure [87]. Consumer preference for such products depends on various properties of the product, including, appearance, odor, extrudability (when applicable), spreadability, adhesiveness and residual greasiness after application. [88] [89]

The visual control of base formulations was performed through the formulations presented in figure 12 and the organoleptic characteristics are shown in table 12.

<table>
<thead>
<tr>
<th>Control</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogeneity</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Odor</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Color a)</td>
<td>W</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>pH</td>
<td>≈ 6</td>
<td>≈ 6</td>
<td>≈ 6</td>
</tr>
</tbody>
</table>

0 (bad), + (moderate), ++ (good), +++ (best) † † Y – Yellow; W - White

The three base formulations (F1, F2, and F3) presented a homogeneous aspect, a similar odor and pH result but a different color. The F1 demonstrates a white color and the F2 and F3 formulations show a yellow coloration due to the calendula oil presence.
Results and discussion

Since the F3 formulation includes half of the calendula oil concentration when compared with F2, the yellow is slightly lighter (table 12).

![Graph showing firmness and adhesiveness of the formulations evaluated (mean values SD, n = 3).](image)

**Figure 13** Results of firmness and adhesiveness of the formulations evaluated (mean values SD, n = 3)

The acceptability and efficacy of topical products require, among other characteristics, that they have optimal mechanical properties (firmness) and appropriate adhesion. [89]

The sensation produced by the application on the skin is one of the most important properties of cutaneous products in addition to the long-term physical stability and physiological effect.

The force necessary to attain a given deformation is called firmness, and in texturograms, firmness is correlated with a positive area (maximum force) whereas adhesiveness is correlated to the negative area. Adhesiveness is regarded as the work necessary to overcome the attractive forces between the surface of the sample and surface of the probe with which the sample comes into contact.

F1 and F2 preparations were similar for adhesiveness and firmness but F3 preparation showed a higher firmness and less adhesiveness that the latest preparations (figure 13).

This can be explained by the increase of carbopol® 980 concentration. Since gel firmness or adhesiveness is directly correlated to the polymer concentration, it was increased in order to improve product robustness and product handling during processing. [90]
Rational design of topical formulations for hand-foot syndrome management

Results and discussion

For F3 formulation propylene glycol was used instead of glycerin in order to facilitate the carvedilol dissolution. However, it was expected that its humectancy ability also would prevent the escape of moisture or water.

The three preparations have comparable evaporation rates with a loss of approximately 60% in the first 5 min.. After 60 min. the film/residue of the formulation may still contain moisture and about 30% of water. Therefore, no impact of propylene glycol on water retention was verified. The petrolatum prevails as a control, showing its barrier capacity (100% of water) (figure 14).

![Figure 14 Percentage of residue for base formulations (F1, F2, and F3)](image)

After centrifugation, all preparations remained homogenous and without phase separation (figure 15).

![Figure 15 Centrifugation results for base formulations (F1, F2 and F3)](image)

Overall, it was decided to move forward with the F3 formulation as the base for the development of the following drug product and cosmetic formulations.
3.2.3. Bioactivity tests

The bioactivity of diclofenac sodium, enoxolone and calendula oil was already assessed, specifically their anti-inflammatory effect, as demonstrated in the introduction section of this work. However, literature shows that in a model of mouse leukemic monocyte macrophage cell line (RAW 264.7) stimulated with the strong pro-inflammatory stimulus LPS only diclofenac sodium [91] and enoxolone [92] [62] anti-inflammatory activity have been studied.

The experimental support for the diclofenac-dependent attenuation of the immune response, including impairing migration and accumulation of leukocytes and diminishing NO production by macrophages, is growing [91] [93] Villalonga et al, tested the concentrations 15 µM and 15 µM and showed the decrease of iNOS levels in Raw 264.7 cells, impairing their activation in response to LPS. However, some discrepancies in the NSAID concentrations used are being raised. For example, Liu et al. [94] used 100 µM diclofenac, whereas some studies have been demonstrated that clinically available doses of diclofenac reach a maximum concentration of 10 mg/ml in blood, which is equivalent to 30 µM [95] Higher doses (150 µM) cause cell death, thus making it impossible to perform functional studies (data not shown). [91]

In this dissertation, it was tested concentrations between 1 µM and 70 µM of diclofenac sodium, to achieve the referred premises. For the tested concentrations, the active ingredient did not compromise the cellular viability of macrophages (Figures 16A). The diclofenac sodium showed a dose-dependent NO inhibition of 50% attained at the concentration of 70 µM (Figure 16B) and the concentration exhibiting anti-inflammatory activity also presented a safety profile to macrophages (Figure 16A and 19A). Meanwhile, at lower concentrations (5, 10, 25 and 35 µM), the diclofenac sodium did not present a significant anti-inflammatory effect.
Results and discussion

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Figure 16 Cell viability and anti-inflammatory activity of diclofenac sodium. (A) Metabolically active cells assessment using a resazurin bioassay. Results are expressed as a percentage of resazurin reduction relative to the control (Ctrl); (B) Anti-inflammatory activity measurement, considering the inhibition of NO production, quantified by the Griess assay. Each value represents the mean ± SEM from one to three independent experiments.

Despite DMSO has a narrow therapeutic concentration range between efficacy as an anti-inflammatory agent and cytotoxicity to the cells that produce the bulk of the cytokines/chemokines (DMSO represses human cytokine/chemokine production), in this work, DMSO did not show significant influence in the anti-inflammatory activity or in the cell viability.

In these experiments, only for the higher concentration of 70 µM (which presents 0.7% of DMSO), a residual anti-inflammatory effect was observed (approx.. 15%) when tested alone (0.7% v/v).
Despite the lack of studies of the enoxolone anti-inflammatory effect in the RAW 264.7 cells, it has been demonstrated that extracts of this compound at low concentrations (20 µM) inhibited LPS-induced nitric oxide release in RAW 264.7 cells. [96, 97]

Throughout this work, a range of concentrations between 4 µM and 40 µM of this active ingredient were tested and it is demonstrated that enoxolone did not compromise the cellular viability of macrophages (Figures 17A).

The enoxolone showed a dose-dependent NO inhibition of 15% attained at the concentration of 40 µM (Figure 17B) and the concentration exhibiting anti-inflammatory activity also presented a safety profile to macrophages (Figure 17A and 19B). Meanwhile, at lower concentrations (< 20 µM), the enoxolone had no significant inhibitory effect, contradict the demonstrated by Zhou et al.

**Figure 17** Cell viability and anti-inflammatory activity of Enoxolone. (A) Metabolically active cells assessment using a resazurin bioassay. Results are expressed as a percentage of resazurin reduction relative to the control (Ctrl); (B) Anti-inflammatory activity measurement, considering the inhibition of NO production, quantified by the Griess assay. Each value represents the mean ± SEM from at one to three independent experiments.
Results and discussion

Rational design of topical formulations for hand-foot syndrome management

For Calendula oil a concentration range of 16 µL/mL to 147 µL/mL (3.2 to 29.4 µL of Calendula oil) was added to cells and did not compromise the cellular viability of macrophages (Figure 18A and 19C). The Calendula oil showed a dose-dependent NO inhibition of 50% attained with 147 µL/mL (Figure 18B). Additionally, with concentrations higher than 49 µL/mL the anti-inflammatory activity presents the higher statistical significance.

**Figure 18** Cell viability and anti-inflammatory activity of Calendula oil. (A) Metabolically active cells assessment using a resazurin bioassay. Results are expressed as a percentage of resazurin reduction relative to the control (Ctrl); (B) Anti-inflammatory activity measurement, considering the inhibition of NO production, quantified by the Griess assay. Each value represents the mean ± SEM from three independent experiments.

Based on the results of an independent sample t-test it is possible to conclude that there were statistically significant differences between the group test (calendula oil) and control (LPS). In the Griess assay, calendula oil at concentrations of 16 and 147 µL/mL showed p<0.05 (p=0.007 and p=0.040, respectively). Additionally, in the cytotoxic assay against RAW 264.7 cells, calendula oil with 16 and 147 µL/mL showed p>0.05 (p=0.07 and p=0.108, respectively) which means that there were no statically significant differences between treatment group and control.
Calendula oil showed anti-inflammatory activity and despite it was not the aim of this work to determine exactly which components are responsible for this bioactivity, the literature attribute this bioactivity to the triterpenoids [98].

Furthermore, the microscopic observation also demonstrates that the cell remains viable when exposed to diclofenac sodium, enoxolone or calendula when in comparison with the control (raw cells 264.7 without any compound addition). No morphological changes were observed (figure 19).

**Figure 19** Microscopic observation of cells (RAW 264.7) treated with diclofenac sodium (60 µM; A), enoxolone (40 µM; B) and calendula oil (147 (µl/ml); C); D – control.
3.2.4. Topical drug product formulations

The visual control of drug product formulations was performed through the following figures.

**Figure 20** First drug product formulations (F3 A\textsubscript{DC/CD}, F3 B\textsubscript{DC/CD+AAG} and F3 C\textsubscript{DC/CD+AAG})

As already described, in the visual inspection the three drug product formulations (F3 A\textsubscript{DC/CD}, F3 B\textsubscript{DC/CD+AAG} and F3 C\textsubscript{DC/CD+AAG}) showed agglomerates and drug substances not dissolved or dispersed. Since too many difficulties in the carvedilol dissolution were faced and so this drug substance was removed from the composition (figure 20).

**Figure 21** Drug product formulations without carvedilol (F3\textsubscript{DC+AAG (1)}, F3\textsubscript{DC+AAG (2)} and mixture of Acid Ascorbic 2-Glucoside and Sodium hyaluronate (AAG+AH))

However, the two replicates of the formulation containing only diclofenac, but still AAG, shows several agglomerates. Furthermore, the addition of AAG to AH led to a sticky gel formation which managed to the exclusion of the antioxidant from the formulation (figure 21).
Results and discussion

Rational design of topical formulations for hand-foot syndrome management

Table 13. Organoleptic controls and pH of prepared drug product formulations (F3 A \textsubscript{DC/CD}, F3 B \textsubscript{DC/CD+AAG}, F3 C \textsubscript{DC/CD+AAG}, F3 \textsubscript{DC+AAG} (1) and F3 \textsubscript{DC+AAG} (2))

<table>
<thead>
<tr>
<th>Control</th>
<th>F3 A \textsubscript{DC/CD}</th>
<th>F3 B \textsubscript{DC/CD+AAG}</th>
<th>F3 C \textsubscript{DC/CD+AAG}</th>
<th>F3 \textsubscript{DC+AAG} (1)</th>
<th>F3 \textsubscript{DC+AAG} (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogeneity</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Odor</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Color \textsuperscript{a)}</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>pH</td>
<td>≈ 6</td>
<td>≈ 6</td>
<td>≈ 6</td>
<td>≈ 6</td>
<td>≈ 6</td>
</tr>
</tbody>
</table>

0 (bad), + (moderate), ++ (good), +++ (best) \textsuperscript{a)} Y – Yellow, LY – Light Yellow; W – White.

The homogeneity of these preparations was bad (F3 A \textsubscript{DC/CD}, F3 B \textsubscript{DC/CD+AAG}, F3 C \textsubscript{DC/CD+AAG}) or moderate (F3 \textsubscript{DC+AAG} (1) and F3 \textsubscript{DC+AAG} (2)) which corroborate the previous visual assumptions (table 13).

Figure 22 Results of firmness and adhesiveness of the drug product formulations evaluated (mean values SD, n = 3)

The firmness and adhesiveness results for F3A \textsubscript{DC/CD}, F3B \textsubscript{DC/CD+AAG}, F3C \textsubscript{DC/CD+AAG} and F3\textsubscript{DC+AAG} (considering the introduction of AAG and the consequent pH change) were significantly different between the tested drug product formulations. F3C \textsubscript{DC/CD+AAG} was the formulation with lower firmness and higher adhesiveness in contrast with F3A \textsubscript{DC/CD} which presented higher firmness and lower adhesiveness (figure 22).

F3\textsubscript{DC+AAG} seems to have similar spreadability characteristics to the chosen base formulation (F3).
Results and discussion

Rational design of topical formulations for hand-foot syndrome management

Figure 23 Percentage of residue for drug product formulations (F3A DC/CD, F3B DC/CD+AAG, F3C DC/CD+AAG and F3 DC+AAG)

As observed in the figure 23, F3A DC/CD, F3B DC/CD+AAG, F3C DC/CD+AAG and F3 DC+AAG preparations have some variability in evaporation rates. A loss approximately of 30%, 40%, 20% and 20% was observed in the first 5 min., for F3A DC/CD, F3B DC/CD+AAG, F3C DC/CD+AAG and F3 DC+AAG, respectively. After 60 min. the film/residue of the formulation may still contain moisture and about 40% and 50% of water, respectively (figure 23).

Despite the observed agglomerates, the physical stability study did not show phase separation (figure 24).

Figure 24 Centrifugation results for base formulations (F3A DC/CD, F3B DC/CD+AAG, F3C DC/CD+AAG and F3 DC+AAG)

Considering the presented results, mainly the non-homogeneous aspect, the initial composition and preparation method were challenged and an optimized emulgel was prepared. The formulation designated as MED included diclofenac as drug substance and the visual control of the three replicates is shown in the following figure.
Results and discussion

Rational design of topical formulations for hand-foot syndrome management

Figure 25 Optimized drug product formulations (MED1, MED2 and MED3)

Table 14. Organoleptic controls and pH of prepared formulations

<table>
<thead>
<tr>
<th>Control</th>
<th>MED1</th>
<th>MED2</th>
<th>MED3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogeneity</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Odor</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Color a)</td>
<td>LY</td>
<td>LY</td>
<td>LY</td>
</tr>
<tr>
<td>pH</td>
<td>Average: 6.25</td>
<td>6.19</td>
<td>6.30</td>
</tr>
</tbody>
</table>

0 (bad), + (moderate), ++ (good), +++ (best) a) Y – Yellow, LY – Light Yellow; W – White.
pH = average of three measurements.

All replicates (MED1, MED2 and MED3) were homogeneous with similar organoleptic characteristics and pH results. The odor was considered best, the color was light yellow and pH approximately 6, which is the desired (table 14).

Figure 26 Results of firmness and adhesiveness of the formulations evaluated (mean values SD, n = 3)

Regarding adhesiveness and firmness, MED2 and MED3 preparations seem more similar between them and MED1 preparation showed slightly higher values of adhesiveness and lower firmness that the other two preparations. However, MED2 and MED3 only differ from MED1, approximately, 30% and 20%, respectively (figure 26).
Results and discussion

Rational design of topical formulations for hand-foot syndrome management

Figure 27 Percentage of residue for drug product formulations (MED1, MED2 and MED3).

A loss of approximately 40%, 30% and 50% was observed in the first 5 min., for MED 1, 2 and 3, respectively. After 60 min. the film/residue of the formulation may still contain moisture and about 40%, 30% and 20% of water, respectively (figure 27). The quick evaporation observed could be beneficial as it will increase the drug’s concentration on the skin surface, which promotes its absorption. The lower residue present in the optimized drug product formulation promotes better topical application conditions, increases the therapeutic adhesion and allows moisturizing the skin without leaving a greasy residue.

Figure 28 Centrifugation results for base formulations (MED1, MED2 and MED3)

After centrifugation, all optimized drug product formulations were homogenous and without phase separation, which demonstrated the formulations of physical stability (figure 28).
Results and discussion

Rational design of topical formulations for hand-foot syndrome management

3.2.5. Topical cosmetic product formulations

The visual aspect of cosmetic product is showed in the following figure.

![Figure 29 Appearance of cosmetic formulations (COSM1, COSM2 and COSM3).](image)

All replicates of cosmetic formulations (COSM1, COSM2 and COSM3) were light yellow and presented a homogeneous appearance and no smell. Additionally, all pH results were approximately 5.0 at preparation time and three days after (table 15).

**Table 15 Organoleptic controls and pH of prepared formulations**

<table>
<thead>
<tr>
<th>Control</th>
<th>COSM1</th>
<th>COSM2</th>
<th>COSM3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogeneity</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Odor</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Color&lt;sup&gt;a)&lt;/sup&gt;</td>
<td>LY</td>
<td>LY</td>
<td>LY</td>
</tr>
<tr>
<td>pH</td>
<td>T0 – $\text{Av} = 5.30$</td>
<td>T0 – $\text{Av} = 5.31$</td>
<td>T0 – $\text{Av} = 5.19$</td>
</tr>
<tr>
<td></td>
<td>SD = 0.006</td>
<td>SD = 0.006</td>
<td>SD = 0.006</td>
</tr>
<tr>
<td>T3 – $\text{Av} = 5.10$</td>
<td>T3 – $\text{Av} = 4.98$</td>
<td>T3 – $\text{Av} = 5.06$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD = 0.023</td>
<td>SD = 0.057</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a)</sup>LY – Light Yellow.

0 (bad), + (moderate), ++ (good), +++ (best)

pH = average of three measurements.

![Figure 30. Results of firmness and adhesiveness of the formulations COSM1, COSM2 and COSM3 (mean values SD, n = 3).](image)
Results and discussion

COSM1 and COSM2 preparations were similar for adhesiveness and firmness but COSM3 preparation showed less firmness and higher adhesiveness than the last preparations. The firmness result of COSM3 varies, approximately, 30% from COSM1 and COSM2 firmness and, approximately, 45% considering adhesiveness (figure 30).

A fourth formulation should be performed and tested in order to ensure the suitability/optimization the preparation method and to confirm the observed characteristics.

Figure 31. Percentage of residue for drug product formulations (COSM1, COSM2 and COSM3)

The three replicates have comparable evaporation rates with a loss of approximately 20% in the first 5 min. However, after 60 min. the film/residue of the formulation may still contain moisture and about 30% of water (figure 31). Despite the faster evaporation would improve the contact of the active ingredient with skin and maybe facilitate its absorption, the slow evaporation, observed in the first minutes, can also be beneficial, as it will increase the percentage of water in contact with the skin. Since one of the aims of this preparation is to prevent symptoms like xerosis, the water content increase can be an advantage.

Figure 32 Centrifugation results for base formulations (COSM1, COSM2 and COSM3)

After centrifugation, all preparations remained homogenous and without phase separation (figure 32).
4. Conclusions

The main goal of any anticancer therapy is a satisfactory outcome for the patient. The reduction or interruption of this therapy can adversely affect patient by the interruption of a potentially life-sparing therapy. Oncological therapies have become more selective. However, cutaneous side effects are common and may worsen the QoL of these patients. HFS is a very common cutaneous adverse event of capecitabine and it is characterized by symmetrical paresthesia, along with desquamative erythema edema, xerosis and pain. [99]. For the purpose of this dissertation, the formulated emulgel (drug and cosmetic products) intended to ensure patient preference and expectations. The findings show that the emulgel incorporating diclofenac sodium (MED formulation) presented balanced firmness and adhesiveness results leading to a product with desired characteristics, good firmness and low residue. This formulation shows homogeneity, pleasant odor and color (light yellow) and a pH result of, approximately, 6. After 60 min. the film/residue of the formulation may still contain moisture and about 30% of water and, thus, promoting better topical application conditions, increasing the therapeutic adhesion and allowing moisturizing the skin without leaving a greasy residue. Its physical stability is good and predicts the quality, safety and efficacy of the product. The cosmetic product containing enoxolone and calendula oil as active ingredients (COSM formulation) seem also to have similar characteristics but a fourth formulation should be prepared to ensure the suitability/reproducibility of preparation method and to confirm the observed physical attributes. The following step would be the study of the rheological behavior, chemical characterization and stability. The stability tests would comprise assessment of: i) stability and physical integrity of products under appropriate conditions of storage, transport and use; ii) chemical stability; iii) microbiological stability; iv) the compatibility between the product and packaging employed. [100] Additionally, also in vivo animal studies and sensory perception tests should be conducted. It was also demonstrated the advantage of the diclofenac sodium used in the drug product and the enoxolone and calendula oil in the cosmetic formulation. The results of this study indicated that these compounds, effectively, possess good potential as anti-inflammatory agents and it was showed that they present a safety profile since no alterations on this type of cell viability were detected. However, further studies may be required to ascertain any possible influence of associations in anti-inflammatory activity.
and its cell viability.
Nevertheless, and envisaging a formulation it will of almost importance to disclose the safety profile of these chemicals on other skin cell types, for instance, keratinocytes and fibroblasts and extend the anti-inflammatory activity to other pro-inflammatory mediators such as cytokines, NF-Kb, among other.
Overall, in the present study, an attempt was made to formulate two different emulgel for topical delivery incorporating: i) diclofenac sodium or ii) enoxolone and calendula oil. The compounds used showed anti-inflammatory activity and no cytotoxicity. MED and COSM formulations showed promising results with respect to the mentioned tests and the work purpose which allows concluding that both preparations were successfully formulated. Consequently, it is an encouraging start for process standardization for HFS management.
5. References


References


Rationale for the design of topical formulations for hand-foot syndrome management


References


References


81. L. Samba Siva Rao, P.M.a.K.V.P., Development and validation of stability indicating method for the quantitative determination of carvedilol and its related


ANNEXES
## ANNEX I

**Table 16** Raw materials and reagents.

<table>
<thead>
<tr>
<th>Excipients</th>
<th>Origin/Batch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Hyaluronate</td>
<td>Guinama 0070494</td>
</tr>
<tr>
<td></td>
<td>Acofarma 170757-G-2</td>
</tr>
<tr>
<td>Carbopol® 980</td>
<td>Noveon EC6D3CC072</td>
</tr>
<tr>
<td>Calendula oil</td>
<td>Acofarma 180210-P-1</td>
</tr>
<tr>
<td>Almond oil</td>
<td>Acofarma 161952-P-1</td>
</tr>
<tr>
<td>Tween® 80</td>
<td>Acofarma 131365-P-1</td>
</tr>
<tr>
<td>Glycerin</td>
<td>Acofarma 171471-P-1</td>
</tr>
<tr>
<td>Paraben stock solution</td>
<td>Methyl Paraben- 1162514</td>
</tr>
<tr>
<td></td>
<td>Propyl Paraben - 1162546</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>Acofarma 150644-P-2</td>
</tr>
<tr>
<td>Triethanolamine</td>
<td>Acofarma 162008-P-1</td>
</tr>
<tr>
<td>Acid Ascorbic 2-Glucoside</td>
<td>Acofarma 172318-P-1</td>
</tr>
<tr>
<td>Active Pharmaceutical Ingredient</td>
<td>Origin/Batch</td>
</tr>
<tr>
<td>Carvedilol</td>
<td>Acofarma 160517</td>
</tr>
<tr>
<td>Diclofenac Sodium</td>
<td>Acofarma 141914-G-12</td>
</tr>
<tr>
<td>Enoxolone</td>
<td>Acofarma 170916-G-1</td>
</tr>
<tr>
<td>Reagents</td>
<td>Origin/Batch</td>
</tr>
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<td>Resazurin sodium salt</td>
<td>Sigma MKBNA870V</td>
</tr>
<tr>
<td>Sulfanilamide</td>
<td>Sigma SZBD0780V</td>
</tr>
<tr>
<td>N-(1-Naphthyl)ethylenediamine dihydrochloride</td>
<td>Sigma MKCB1054V</td>
</tr>
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</table>
### ANNEX II

**Table 17** Base formulations identification

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Abbreviation</th>
<th>Identification according with the presence of almond and/or calendula oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base</td>
<td>F</td>
<td>F1 – only almond oil</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F2 – only calendula oil</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F3 – both almond and calendula oil</td>
</tr>
</tbody>
</table>

**Table 18** Drug product formulations identification

<table>
<thead>
<tr>
<th>Formulation (with diclofenac and carvedilol)</th>
<th>Abbreviation</th>
<th>Identification according with the presence of Acid Ascorbic 2-Glucoside (AAG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug product</td>
<td>F3 A&lt;sub&gt;DC,CD&lt;/sub&gt;</td>
<td>F3 A&lt;sub&gt;DC,CD&lt;/sub&gt; absence of AAG</td>
</tr>
<tr>
<td>Drug product (only with diclofenac)</td>
<td>F3&lt;sub&gt;DC&lt;/sub&gt;</td>
<td>F3 B&lt;sub&gt;DC,CD,AAG&lt;/sub&gt; 1% of AAG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F3 C&lt;sub&gt;DC,CD,AAG&lt;/sub&gt; 2% of AAG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F3&lt;sub&gt;DC,AAG&lt;/sub&gt; 2% of AAG</td>
</tr>
</tbody>
</table>

**Table 19** Identification of optimized drug product formulations

<table>
<thead>
<tr>
<th>Formulation (only with diclofenac)</th>
<th>Abbreviation</th>
<th># of replicate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug product</td>
<td>MED</td>
<td>MED 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MED 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MED 3</td>
</tr>
</tbody>
</table>

**Table 20** Identification of cosmetic formulations

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Abbreviation</th>
<th># of replicate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cosmetic product</td>
<td>COSM</td>
<td>COSM 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>COSM 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>COSM 3</td>
</tr>
</tbody>
</table>
Diva Silva

Rational design of topical formulations for hand-foot syndrome management

Faculdade de Farmácia