Ointments:

towards the understanding of structure, stability and processing

Anton Joris Pancras van Heugten

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Ointments: towards the understanding of structure, stability and processing

Zalven: vergaren van kennis van de structuur, stabiliteit en productie

(met een samenvatting in het Nederlands)

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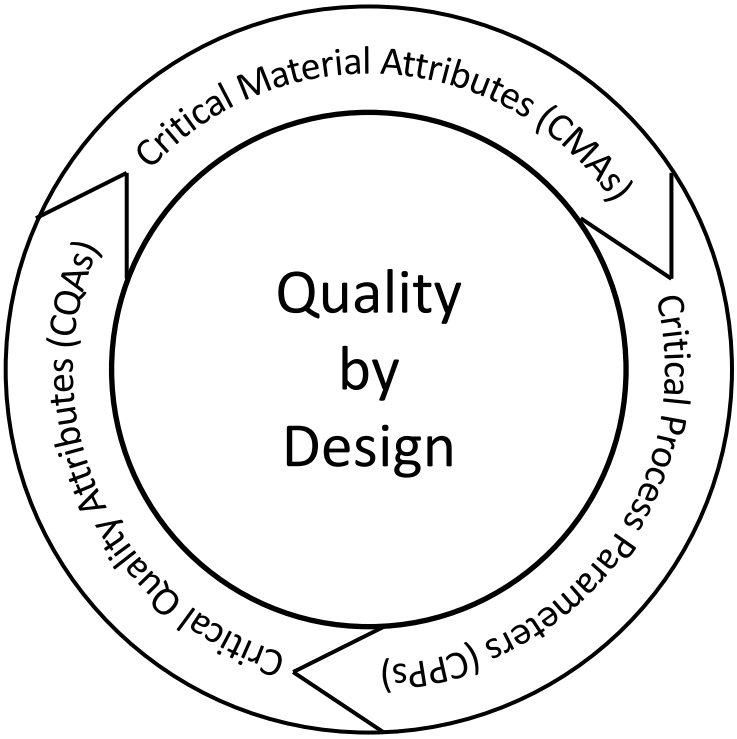
Introduction



Introduction

Dermatological products: setting the stage

The majority of the research on the fundamental physical characterization of dermatological products and excipients was conducted before the 1980's. Since then, the arsenal of analytical techniques has been significantly improved. Despite this increase in investigational possibilities, there is hardly any recent innovation in dermatological products. It is expected that a more thorough understanding of the physical properties of excipients and formulations will eventually lead to dermatological product innovation. Furthermore, in the past decades a paradigm shift has taken place within the pharmaceutical industry from a one-factor-at-a-time experimentation strategy to a more comprehensive, statistical and science based approach towards product development: Quality by Design. This applies to the production of dermatological products as well but has not yet been fully implemented in the pharmaceutical industry.

Additionally, the requirements of authorities on the degree of chemical drug degradation have increased (1). For example, recently triamcinolone acetonide ointment ("0.1% TCA zalf FNA") was withdrawn from the Dutch market due to these increased requirements (2). This shows that more understanding of the chemical degradation processes in dermatological product is needed.

The consequence of all the above described developments is that a higher degree of product and process understanding is demanded for dermatological products. This requires more thorough biopharmaceutical research to be conducted on these products and production processes.

Ointments: a historical perspective

This dissertation focuses on ointments. The use of ointments has been traced back to the Ancient Egypt, where these were applied in ritual balms, cosmetics and pharmaceuticals. **Figure 1** shows a timeline of the development of ointment formulations. A review on the composition of Egyptian mummification balms and Roman unguents shows that ingredients still used today such as animal fat, vegetable oil and beeswax were used in these cosmetics (3). Unguent jars have been traced back to 2500 BC. Around 1500 BC, a medical papyrus of large significance to the historical development of pharmacy was written: the Ebers papyrus. This document contains a collection of recipes of 811 prescriptions and some 700 drugs. Interestingly, in this

papyrus not only the ingredients but also descriptions of how to prepare the medicine are presented, including suppositories and ointments. Later on, from 460 BC the Greeks, in particular Hippocrates, further developed the practice of medicine. In the writings of Hippocrates and his scholars from the Hippocratic school, significant advancement of the level of pharmaceutical skill is described. These documents also describe ointments. Interestingly, the Greek defined pharmacists who made ointments as *myropoeos* or *myrepsos* (meaning: maker of ointment; *myron* = ointment). Galen further advanced all previous work between AD 130 – 200 in the Roman Empire. He extensively built on the previous work and laid out his concepts in his *Methodo medendi (On the Art of Healing)*. These dominated pharmacy until Paracelsus challenged them in the sixteenth century. Galen defined the preparation of drugs using multiple ingredients, today Galenic formulation deals with the compounding of active ingredients. Fundamentally, it can be stated that Galenic formulation is the transformation of an active ingredient by mixing it with other ingredients to formulate a dosage form. In the sixteenth century the first pharmacopoeias were published, thereby structuring all previous work and providing a handbook to pharmacists for the preparation of drugs. In 1714 Daniel Turner's *De morbis cutaneis* was published which can be considered the founding text of British dermatology, including descriptions of how externally applied medicines enter the body through pores in the skin. In 1869 pharmaceuticals were prepared on an industrial scale for the first time by the pharmacist Eugen G.H.W. Dieterich in Germany (4). The Formularium Nederlandse Apothekers (FNA) was released in 1967 which describes standardized formulations for specials. Due to its historical use, most of the ointment formulations are formulated on an empirical basis. This impacts not only their formulation but also the production process.

Since the 1980s hardly any attention has been paid to the study of the physicochemical properties of ointments. The research that mentions ointments after the 1980's generally focuses on release or other surrogate parameters that do not elucidate the fundamental physicochemical characteristics, such as consistency or stability. Nowadays, the majority of skin related research focuses on other areas, such as: skin morphology (5), penetration enhancers (6), nano- and microemulsions (7), microneedles (8), modeling the skin barrier (9,10), liposomes, transferosomes or other similar types of formulations (11). Although all these research areas are relevant, the overwhelming majority of dermal products still consists of ointments and creams. The wide arsenal of currently available new techniques will shed new light on the physicochemical characteristics of ointments. Because of this, the study of the consistency and stability of ointments is relevant.

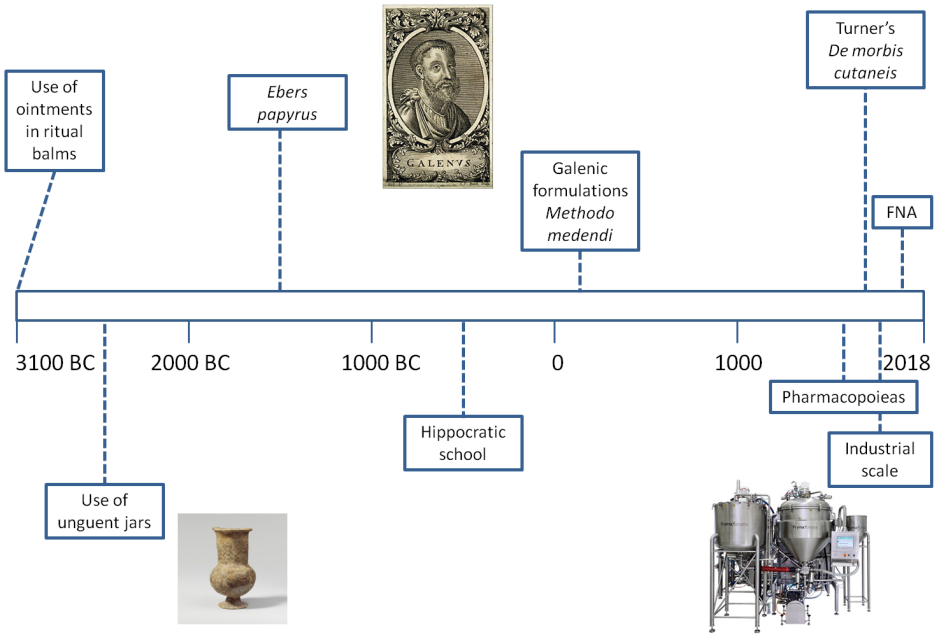


Figure 1. Historical evolution of ointments from its ritual use in 3100 BC to the industrial scale production of the 21st century.

Types of topical formulations

Topical formulations appear in a number of different forms. The majority includes semi-solid formulations, namely: ointments, creams, gels and pastes (Ph. Eur. Monograph 0132). To summarize this, an overview of the different formulation categories is presented in **Table 1**. Although different categories are described in the Pharmacopoeia, the distinction between these categories on the basis of their definition is not always clearly defined. For example, the definitions for ointments and creams overlap (**Table 1**). Evidently, when a liquid is dispersed in a single-phase base we in fact end up with a multiphase basis. Even a commonly used product in the Netherlands, Koelzalf FNA, is an example of a questionably defined ointment. This "cooling ointment" (translation of "Koelzalf") contains 25% water. Obviously, when the definitions are strictly followed, Koelzalf FNA is in fact a cream and not an ointment. A new proposal for defining ointments and creams will be made at the end of this dissertation.

As mentioned in **Table 1**, ointments behave non-Newtonian and show viscoelastic properties. This means that when stress is exerted onto an ointment the viscosity is dependent on the amount of shear applied. Viscoelastic means that ointments behave

solid-like at rest and liquid-like when moved (12). Consequently, the physicochemical characteristics of ointments are of great importance, since these will define its behavior not only during production but also when applied to skin (13).

Production of ointments

As mentioned in section 1.2, the development of formulations and production processes is highly empirical. Traditionally, ointments are produced using mortar and pestle for individual patients and in vacuum controlled stirring vessels on industrial scale, as is shown in **Figure 2**. These two scales of production are vastly different; e.g. in the dimensions, degree of shear forces applied, temperature control, etcetera. Therefore, increasing the scale of production or upscaling is a major challenge for pharmaceutical industry. In order to successfully scale up a process the similarity principle is adopted, which assumes that across all equipment and process scales equal ratios between for example dimensions, forces and temperature gradients should be achieved (14). Here, often dimensionless numbers are used as expression of these ratios. In practice, it is impossible to fully meet the requirement of similarity and therefore scale up is a serious point of attention in drug development (15). One way to approach the upscaling of a process is to do this as much as possible on the basis of process understanding, which is basically the paradigm underlying Quality by Design (QbD) (16). The idea is that one should know which parameters can be ignored and which cannot. The knowledge of the critical parameters that contribute to the final product specifications (critical quality

Table 1. Overview of the different topical formulations described in the Pharmacopoeia for application to the skin.

	Ointments	Creams
Description	Mostly “single phase” but may contain small quantities of liquids or powders	Multiphase systems consisting of a lipid and an aqueous phase
Typical formulation characteristics	• (Usually) free of water • Consists of paraffins and/or fats • Apolar environment • May contain liquid and solid macrogols (hydrophilic ointments) • May contain emulsifiers (water-emulsifying ointments)	• Emulsions of oil in water (water is the continuous phase) • Or emulsions of water in oil (oil is the continuous phase) • Partly polar environment
Typical rheological properties	Show viscoelastic properties and are non-Newtonian	Show viscoelastic properties and are non-Newtonian

attributes (CQAs)) enables the selection of the appropriate settings at larger scale. Normally, the assessment of critical process parameters is conducted on lab scale level since experiments at industrial scale batches are associated with high costs (17).

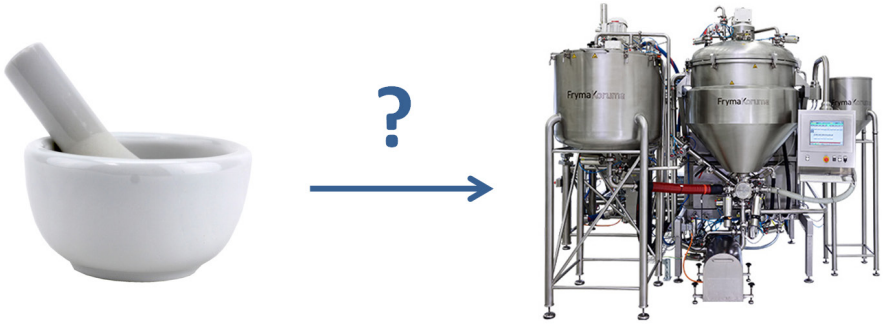


Figure 2. Increase of scale of production from individual preparations to industrial manufacturing.

Quality by Design

In the pharmaceutical industry, complying with the Good Manufacturing Practices (GMP) is mandatory. Until 2000, products were defined by the manufacturing process and quality was based on empirical analysis of characteristics that may or may not entirely matter. Generally, the processes and products were not well understood. This led to low manufacturing efficiencies, higher costs and shortages in various products. Regulators such as the Food and Drug Administration (FDA) were perceived to be the cause of these problems. Therefore, at the end of 2002 the FDA kicked off an initiative to

	Gels	Pastes
	Liquids gelled by means of a suitable gelling agent	Semisolid preparations containing large proportions of solids finely dispersed in the basis
	• Oleogels consist of lipophilic ingredients such as paraffin, polyethylene or fatty alcohols gelled with colloidal silica or aluminum or zinc soaps • Hydrogels consist of water, glycerol or propylene glycol gelled with poloxamers, starch, cellulose derivatives, carbomers and magnesium-aluminum silicates	• Contain large proportions of dispersed solids
	Show viscoelastic properties and are non-Newtonian	Show dilatant rheological properties

implement changes to the GMP guidelines. Primary goal was to place responsibility for product quality on the pharmaceutical industry itself instead of changes submitted by industry to the FDA for approval. At that time the FDA received changes approximately every 20-30 minutes! This shift in responsibility gave rise to a number of guidelines of which two, released in 2004, were critical (18,19). In these guidelines, the intellectual foundation for implementing QbD was presented. An important factor in this was the risk-based approach, meaning that one started to distinguish on the basis of systematic and scientific understanding what matters the most (i.e. is "critical") in order to focus upon these. This fundamentally changed the perception of the value of in-process measurements and quality control. A general shift from "inspecting-in quality" to "building-in quality" was enforced through these guidelines. In 2008 the pharmaceutical development guideline (Q8) was revised, in which notion was made of a design space (20). With this term, the range of process settings is meant within which product of good quality can be produced. This introduced the need for the application of QbD tools in order to establish such a design space (21,22). By using a QbD approach, well-designed drugs are developed in contrast to the aforementioned Galenic formulations that were empirically developed. Similarly, todays specials are required to meet higher demands. These specials are empirically developed and a QbD approach can provide the necessary product and process understanding to improve the quality of these drug products.

QbD became the way forward towards a more scientific, risk-based and holistic approach to pharmaceutical development for both the FDA and industry. The general goal of QbD is to gain product and process understanding, thereby building quality into the product. Conventional pharmaceutical practices focus on quality control after production, thereby testing quality of the end-products. The first step in QbD is to determine the specifications of the anticipated product; "beginning with the end in mind". This goal is defined as the quality target product profile (QTPP). In this QTPP typically the demands for patient use are included. These are translated to critical quality attributes (CQA's), which may be viewed as the product specifications, such as its content (uniformity), dissolution, stability, etc. Furthermore, a number of attributes towards excipients (critical material attributes (CMA's)), formulation and production process (critical process parameters (CPP's)) are studied for their impact on the CQA's. In other words, by following the systematic approach of QbD both raw materials, formulation and production process are studied thoroughly which leads to more product and process understanding.

This brings us to the challenges in the production of ointments nowadays. These will be discussed from a QbD point of view as is shown schematically in **Figure 3**.

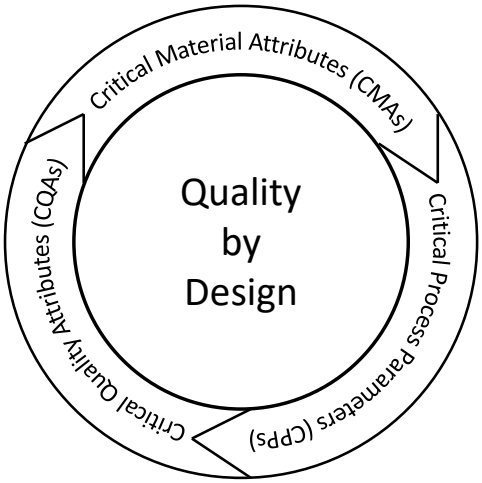


Figure 3. Schematical overview of the Quality by Design (QbD) approach used in this dissertation.

Challenges

Critical material attributes (CMAs)

Both the active pharmaceutical ingredient (API) and the excipients may have certain CMAs that can influence the quality of the end product significantly. Since the majority of excipients that are used in ointments are of natural origin, for example wool fat and petrolatum (white soft paraffin or Vaseline®), these are generally poorly defined and only broad specifications are set in the Pharmacopoeias. Petrolatum is probably one of the most important due to the fact that it is used in the majority of ointments. Of this ingredient large quantities are used worldwide; yearly, approximately 80 million kilograms of petrolatum is used for pharmaceutical products. The same amount is used for cosmetic formulations adding up to a total of 160 million kilograms for topical formulations (cosmetic and pharmaceutical) yearly (23). If we zoom in on the 80 million kilograms used for pharmaceutical formulations and we correct for the density of 0.9 kg/L, staggering comparisons can be made. The total volume of petrolatum used yearly corresponds to 36 olympic size swimming pools or 1317 large sea containers.

Interestingly, there is still need for knowledge with respect to petrolatum. In 1971 an extensive review on petrolatum was published (24). In this review a wide range of attributes of petrolatum is described. For example, two types of petrolatum are used in pharmaceutical applications; yellow and white petrolatum. The only difference is the color; bleaching of yellow petrolatum leads to white petrolatum. Furthermore, it is known to consist of n-paraffins, iso-paraffins and cyclic paraffins. The proportions of these may vary considerably between batches and grades of petrolatum. The rheological properties of petrolatum were shown to vary greatly dependent on temperature and grade differences (25). The differences in rheological properties were attributed to a three dimensional crystalline network which shows a resistance to flow (24,26,27). Of this three dimensional crystalline network no clear evidence exists. Only microscopical studies are described in literature, an example is shown in **Figure 4**.

Critical Process Parameters (CPPs)

As has been discussed before the production of ointments can be challenging. CPPs are involved in product quality and batch-to-batch reproducibility. Also in the scale up of production processes identification of CPPs is important. This generally requires a thorough process understanding of the lab scale production. To acquire this process understanding a design of experiments (DoE) approach can be used. Using such an

approach multiple variables, for example mixing rate, filling temperature, cooling rate, heating temperature, can be varied and the influence of individual variable settings on CQAs can be observed. This therefore is a powerful approach to acquire process understanding.

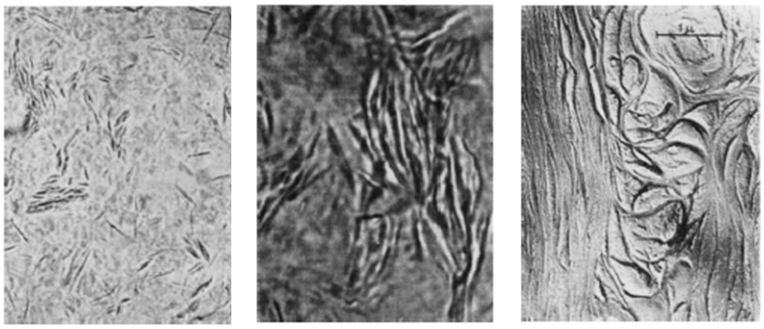


Figure 4. Visualization of petrolatum three dimensional crystalline network at three different magnifications (350 X, 1050 X and 26000 X) as described by (27).

In general, it is well known that during production certain process parameters are more critical for the product CQAs than others. For example for diverse applications such as ophthalmic ointments (28), emulsion stability (29) and Chinese Hamster Ovary cell cultures, DoE approaches acquired the necessary insight into the criticality of parameters on product CQAs. In ophthalmic ointments the mixing rate, temperature, time and cooling rate were CPPs, for emulsion stability the preparation methodology and shear rate and for Chinese Hamster Ovary cell cultures the temperature, osmolality and agitation. For the production of ointments other than ophtalmic DoE studies have not yet been published.

Critical Quality Attributes (CQAs)

For pharmaceutical products CQAs are generally characteristics that are analyzed during quality control after production. Of these CQAs, the stability of the API is one that can be challenging. In general APIs are stable as such; a well-defined crystalline material stored in an appropriate container. However when APIs are mixed with excipients, stability issues commonly occur. Drug-excipient interactions are common, excipients may contain reactive impurities such as reducing sugars, aldehydes, peroxides, metals, nitrate/nitrite and organic acids (30–33).

In topical formulations, corticosteroids are widely used APIs. Corticosteroids are anti-inflammatory agents of the steroid hormone class. These bind in the target cell to specific cytosolic glucocorticoid receptors and subsequently interact with glucocorticoid receptor response elements on DNA thereby altering gene expression (34). The affinity for the glucocorticoid receptor differs for each corticosteroid. Since the 1950's corticosteroids are used for many skin diseases, such as eczema and psoriasis. For these applications, corticosteroids are used in polar environment (creams and lotions) and in non-polar environment (ointments) (**Table 1**).

One of the concerns with corticosteroid containing products is the chemical stability. According to the ICH guideline only limited amounts of degradation products may be present in the formulation (1). Apart from the quantitative specifications of degradation products, their qualification is also important. Degradation can be studied using stress testing, which is described elsewhere extensively (33).

Also the consistency or spreadability of ointments can be considered a CQA. This consistency can be characterized by rheometry and is known to depend on formulation, processing and thermal history (25,28,35). Yield stress is the amount of force needed to make a sample flow and can therefore be considered as a measure for spreadability (26).

General aim of this dissertation

The aim of this dissertation is to acquire a deeper understanding of widely used ointment excipients (CMAs), processing and scale up (CPPs) and stability of corticosteroids in ointments (CQAs).

In chapter 2.1 and 2.2, the CMAs of the excipient petrolatum are studied. Its variable rheological behavior has not yet been explained in literature. Therefore, a thorough structural characterization is conducted to understand why and how rheological differences between and within petrolatum grades exist.

In chapter 3.1 the CPPs for the lab scale production of cetomacrogol ointment are studied using a novel type of DoE. In chapter 3.2 the outcomes of this lab scale study are translated to industrial scale production to study how lab scale process understanding can facilitate the scale up of a production process.

In chapter 4 the CQAs, more specifically the chemical stability and degradation pathways, of corticosteroids are studied in more detail. Most focus (chapter 4.1 and 4.2) lies on the corticosteroid triamcinolone acetonide (TCA) which is widely used in cream and ointment formulations, such as TCA ointment FNA. In chapter 4.3 the findings for TCA are translated to other structurally similar corticosteroids and the big picture of corticosteroid degradation is proposed.

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Chapter 2

Critical Material Attributes (CMAs)

Chapter 2.1

Elucidation of the variability in consistency of Pharmacopoeia quality petrolatum

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Abstract

The Pharmacopoeia monograph for petrolatum poorly defines the material's physical properties. Indeed, differences between petrolatum grades can be substantial; yield stress varies between 65 and 280 Pa which can be compared to the consistency of respectively thin cream or thick ointment.

This variation is not only due to differences in composition or refining process but also as a result of different processing; for example, thermal history influences petrolatum structure considerably. Slow cooling of petrolatum resulted in a yield stress of 26 Pa and fast cooling in 79 Pa. X-ray showed that crystallinity was 0.7% for the first cooling case and 1.5% for the second one. Crystallite size was estimated to be 20-50 nm. To investigate if this relatively small difference in crystallinity may induce the difference in consistency, 15 nm SiO_2 particles were added to petrolatum. Indeed, a small increase in SiO_2 concentration led to a major increase in yield stress. This was argued to be due to the small size of the particles, resulting in a large increase in absolute number of particles.

The Pharmacopoeia does not unambiguously define the pharmaceutical excipient petrolatum. As a consequence, the formulator has to take care of selecting the appropriate grade as well as to carefully control the processing of the material in order to achieve a consistent pharmaceutical product.

Introduction

Petrolatum is one of the most commonly used ingredients in pharmaceutical and cosmetic ointments and creams. Despite the wide application of petrolatum, the Pharmacopoeia monograph (1) for petrolatum or white soft paraffin describes it poorly. Only general identification tests are described and the physical characterization methods are drop melting point and consistency by a penetrometry test. Penetrometry is a method by which relatively large viscous deformations in materials can be observed. However, since petrolatum is a viscoelastic material, meaning it combines both viscous and elastic characteristics, structural testing should also focus on its elastic properties. This can be studied in more detail using oscillatory stress testing (2). Therefore, the Pharmacopoeia monograph is not capable of describing differences between petrolatum grades in great detail and thus possibly a great variety of different petrolatum products comply with the Pharmacopoeia monograph. Similar tests are described in the United States Pharmacopoeia and Japanese Pharmacopoeia.

The chemical composition of petrolatum can be described as a blend of three different components, two solid hydrocarbon waxes and a liquid hydrocarbon fraction (3). The two waxes are described as paraffin and a microcrystalline wax consisting of an average of 26-30 and 41-50 carbon atoms respectively. Furthermore, paraffin waxes contain mainly straight chain alkanes whilst microcrystalline waxes contain iso-alkanes and naphtene-containing alkanes (4). The amounts and nature of these components may be different for various grades of petrolatum (5,6). Sources of crude material, differences in refining steps and differences in blending after refining can all attribute to differences in nature of petrolatum raw materials (2).

Apart from chemical composition, it is known from other fields that thermal history and crystallinity can affect material structure. This is shown in studies on polyethylene glycol (PEG) (7) and medium chain polyhydroxyalkanoate (8). Since petrolatum has been described as a gel structure (3) there may be parallels to polymer structure and behaviour under different thermal conditions.

A method to study the properties of petrolatum is the assessment of the consistency. For example the physical properties of several white petrolatum products of Japanese Pharmacopoeia quality were studied and it was concluded that the spreading properties of the different petrolatum grades differed substantially. Physical properties were defined

by the penetrating stress, shear stress and yield stress (9). Moreover, brand and generic clobetasone butyrate ointments have been studied and it was concluded that different grades of petrolatum were used as indicated by a GC/MS method and it was suggested that these are the cause of different sensory properties (10). Significant differences in rheological properties as a function of temperature were found for three different petrolatum grades (2). However, an elucidation of the cause of the differences in consistency is lacking. Differences in consistency are relevant for the sensory properties of an ointment. It has been shown that rheological characterization yields a good indication for these sensory properties (11) .

Apparently, petrolatum products can exert significantly different rheological properties. This may impact both sensory properties of petrolatum containing products for patients but also the generic applicability of different Pharmacopoeia quality petrolatum grades in manufacturing processes. However, it remains unclear what causes these significant differences. Consequently, a lack of a proper understanding of the physical characteristics of petrolatum exists. Because of this, pharmacists or formulation scientists working in pharmaceutical or cosmetic industry are not able to explain how and why Pharmacopoeia quality petrolatum grades are different.

More understanding of this widely used pharmaceutical excipient is therefore crucial and thus our aim is to describe how Pharmacopoeia quality petrolatum grades can differ and what factors determine their structure.

Materials and methods

Materials

All used materials are shown in **Table 1**. When Ph. Eur. quality is mentioned, this is with reference to the Pharmacopoeia monographs of white soft paraffin (for petrolatum), hard paraffin (for petrolatum waxes) or liquid paraffin (for paraffin oils).

Table 1. Overview of materials used in this study

Ph. Eur. quality petrolatum grades	Snowwhite N®*, Snowwhite A4®*, Snowwhite T5®*, Fonoline H®* and Hansen and Rosenthal KG Pionier 3476®**
Ph. Eur. quality petrolatum waxes	Microcrystalline wax concentrate HLA3129*
Ph. Eur. quality paraffin oils	Kaydol®* and Lytol®*
Other components	Paraffin wax*, Microcrystalline wax* and Aerosil® 200 vv Pharma ***

*: Kindly donated by Sonneborn International, Amsterdam, The Netherlands
**: Hansen and Rosenthal KG, Hamburg, Germany
***: Evonik, Paris, France

Batch production

Petrolatum Snowwhite N® was melted for 10 minutes at 80 °C on a water bath and subsequently filled in 100 ml PP containers. These were stored in a hot air cabinet that was slowly cooled down (approximately 0.1 °C/min) to room temperature, cooled down on a lab table at room temperature (approximately 0.4 °C/min) or cooled down in a freezer at -26 °C (approximately 5 °C/min) for three days. Afterwards, containers were stored at room temperature for two months prior to analysis.

An experimentally produced petrolatum was produced using 40 g microcrystalline wax concentrate HLA3129 and 60 g Lytol® oil. Both components were melted and mixed for 10 minutes at 80 °C on a water bath. Subsequently 50 g was poured in aluminium bags and either quickly cooled (approximately 5 °C/min) between ice plates for 10 minutes or slowly (approximately 0.125 °C/min) to room temperature for several hours.

Batches containing SiO_2 were produced by mixing in parts of the Aerosil® and molten petrolatum. This mixture was subsequently transferred to a 4M8-Trix homogenizer (ProCePt, Belgium) and mixed while cooling down to room temperature at 50 rpm for 30 minutes.

Rheological characterization

A stress-controlled rheometer (TA instruments HR-2 USA) equipped with a step-peltier stage (20 °C) and a 40 mm sandblasted parallel plate (TA-instruments Cone plate geometry 40 mm) was used. Approximately 5 gram of sample was placed on the peltier plate before slowly lowering the upper plate to the preset trimming gap of 1050 µm. After trimming of excessive sample, the geometry gap was set at 1000 µm. Before analysis, samples were equilibrated for 3 minutes at 20 °C.

Yield stress was characterized using an oscillatory stress sweep where a logarithmic stress sweep at a frequency of 1 Hz was conducted within the range of 10 to 2000 Pa. The point of intersection with the G 'and G'' was defined as yield stress.

Viscosity of Kaydol® and Lytol® were estimated at 24.1 °C using a Brookfield DV3T rheometer (AMETEK Brookfield, Ochten, The Netherlands) with a Brookfield EZ-lock spindle set, spindle type 2 operated at 100 rpm.

X-Ray diffractometry

Room temperature XRD measurements were carried out on a Bruker-AXS D8 Advance powder X-ray diffractometer, in Bragg-Brentano mode, equipped with automatic divergence slit and a PSD Våntec-1 detector. The radiation used was Cobalt $Ka_{1,2}$, l = 1.79026 Å, operated at 30kV. A range of 5 – 60 2Θ was studied. Crystallite size was calculated using the Scherrer equation (Equation 1) by using a K-value of 0.89 and calculating the β value by measuring the width of the peak at half the maximum intensity and subtracting the instrumental line broadening.

Equation 1: $$\tau = \frac{K\lambda}{\beta cos\theta}$$

Crystallinity was determined using the ratio between background and peak area, calculated with the XRD analysis program DiffracEVA (Bruker, The Netherlands).

Results and discussion

Rheological differences between grades of petrolatum

For a number of different grades of Ph. Eur. quality petrolatum yield stress was measured. The results are shown in **Figure 1**.

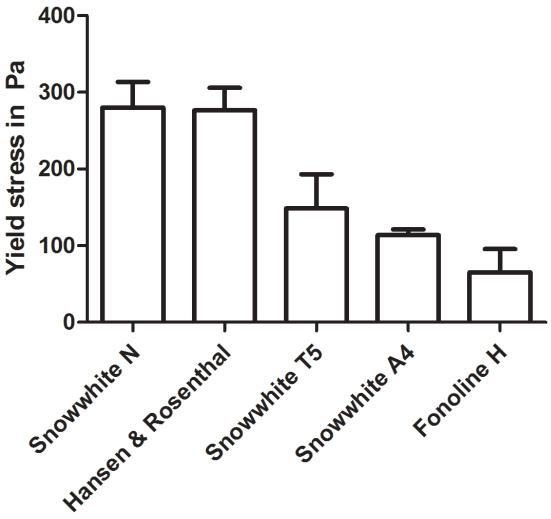

Figure 1. Differences in yield stress in Pascal at 20 °C for different Ph. Eur. quality petrolatum grades, 95% confidence interval is shown in bars.

As can be clearly seen in **Figure 1**, the Pharmacopoeia quality petrolatum grades differ significantly in rheological properties. The most easily spreadable petrolatum (Fonoline H®) has an average yield stress of 65 Pa and the stiffest petrolatum (Snowwhite N®) a yield stress of 280 Pa, which can be considered a profound difference since these result in different sensory sensations. These are comparable to those of a thin cream (65 Pa) and an ointment (280 Pa). Obviously, Pharmacopoeia criteria are not sufficient to select petrolatum with consistent rheological properties. This complicates the choice for the appropriate grade of petrolatum in pharmaceutical or cosmetic manufacturing when there is a need to provide the patient with a constant sensory feel when applying the product.

For petrolatum Snowwhite N® the influence of thermal history has also been tested, results are shown in **Table 2**.

Table 2. Influence of thermal history on yield stress of petrolatum Snowwhite N® shown for four different cooling and storing conditions. After thermal treatment samples were stored for two months at room temperature.

Thermal history	Yield stress at 20 °C (in Pa)
Bulk sample from warehouse	280
Cooled in hot stove cooled with approximately 0.1 °C/min	341
Cooled on lab table cooled with approximately 0.4 °C/min	463
Stored at -26 °C for three days and heated to room temperature afterwards	661

It can be clearly observed that due to differences in thermal history even within a single grade of petrolatum significant differences in consistency exist (ranging from 280 to 661 Pa). These differences are still present after storing the samples for two months at room temperature. Surprisingly, the influence of thermal history has not been described for petrolatum before. Clearly, not only chemical composition but also thermal history influences petrolatum structure significantly. This consequently implicates that even if one grade of petrolatum is used in manufacturing of a pharmaceutical product, batch to batch variety may still be significant depending on thermal history.

Influence of thermal history on crystallinity and structure

Since petrolatum is a saturated solution of different alkane chain lengths (3) alkane solubility will depend on temperature. At lower temperature the solubility of longer chain alkanes will be lower and thus these will solidify and potentially crystallize. Therefore, crystallinity will be depended on thermal history. Two experimentally produced petrolatum samples with a different thermal history were characterized using X-ray diffractometry and results are shown in **Figure 2** and **Table 3**.

A small difference in amount of crystalline material was found between slowly and rapidly cooled petrolatum (0.7% and 1.5% respectively). To determine crystallite size it is conventional to use polarised light microscopy or confocal laser scanning microscopy. However, it appeared that the observed crystallite size is greatly dependent on sample preparation and the part of the sample that is observed using these techniques. Interestingly, crystallite size can also be determined using X-ray. It is a convenient method for determining the mean size of nanosized crystallites and sample preparation is not as destructive as for microscopy. Furthermore, a larger quantity of sample can be observed and these samples are therefore more representative for the material. Crystallite size can be analysed by determining the peak width of the crystalline peaks and calculating the crystallite size using the Scherrer equation (12).

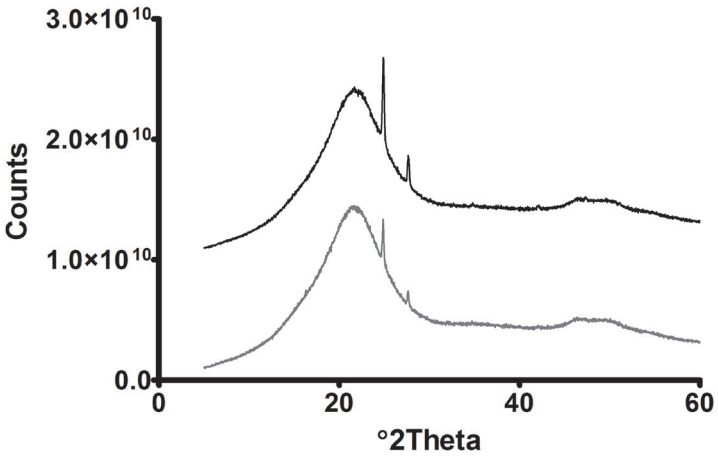

Figure 2. X-ray data of experimentally produced petrolatum with different thermal history, forcefully cooled petrolatum shown in black and slowly cooled petrolatum shown in dark grey. The black line has been moved upwards with an increment of $1x10^{10}$ counts to observe the differences.

Table 3. Influence of thermal history on the yield stress and crystallinity of experimentally produced petrolatum

Thermal history	Yield stress at 20 °C (in Pa)	Crystallinity in %
0.1 °C/min to room temperature	25.5 ±0.8	0.7
5 °C/min to 0 °C and then to room temperature	78.6 ±3.1	1.5

For the experimentally produced petrolatum crystallite size was determined by X-ray diffractometry. Crystallite size was around 30 nm and peaks were at the same position suggesting same crystal type. The resolution of the X-ray diffractometer was insufficient to estimate crystallite size accurately for this small crystallite size. Therefore, only a certainty range of crystallite sizes can be provided of 20-50 nm.

For the samples no clear influence of cooling rate on crystallite size was observed while this influence has been described for other products such as milk fat, lard and canola oil (13,14). Here, it was shown that faster cooling results in smaller crystals. For petrolatum however, crystallite size was similar for both samples. This may be due to the fact that petrolatum is a chemically different product compared to milk fat and canola oil and may therefore crystallize differently. Furthermore, crystal size for milk fat and canola oil was determined using polarised light microscopy, which can observe crystals of micrometer size and not nanometer size. This technique exerts high stresses to samples during preparation as well (4).

Apart from the difference in crystalline mass, also a profound difference in yield stress was found (**table 3**): the 0.7% crystalline petrolatum exhibited a yield stress of 25 Pa at 20 ^{o}C compared to 79 Pa for the 1.5% crystalline material. Apparently, thermal history has a major impact on petrolatum structure. This has also been reported in literature for polymers (7,8) and food products such as milk fat and lard, palm oil and canola oil (13–16).

Linking crystallinity to structure

Because of the significant influence of thermal history on petrolatum yield stress, it was hypothesized that a minor difference in crystallinity can influence yield stress significantly. To study this the addition of particles with a size comparable to the observed crystallites was tested. SiO_2 particles with a primary particle size of 15 nm (Aerosil® 200 VV Pharma) were added to petrolatum Snowwhite N®.

Results are shown in **Figure 3**. It can be clearly seen that above approximately 0.9% of silica particles a significant increase in yield stress of petrolatum occurs. Obviously, a relatively small amount of small particles can have a significant effect on yield stress of petrolatum. From this perspective, it is not unlikely that a difference of 0.8% in crystallinity between the two tested samples may have a profound effect on petrolatum structure.

The steep increase in yield stress after the addition of a certain amount of particles suggests a threshold value. A similar phenomenon has been studied extensively for polymer composites where the influence of particulate filler size was evaluated. It was shown that a particle size dependent influence of polymer composite reinforcement exists. Small particles reinforce the polymer composite to a larger extent than larger particles. Furthermore, it was shown that particle size influences polymer composite reinforcement but also that the influence of amount of material added was non-linear suggesting a threshold value (17).

Since a small increase in crystalline mass of petrolatum crystals will account for a large increase in number of petrolatum crystallites it can be expected that this increase in number of particles can have a profound influence on petrolatum rheological properties. This is in line with literature on polyhydroxyalkanoates where a higher amount of particles results in a harder structure (8).

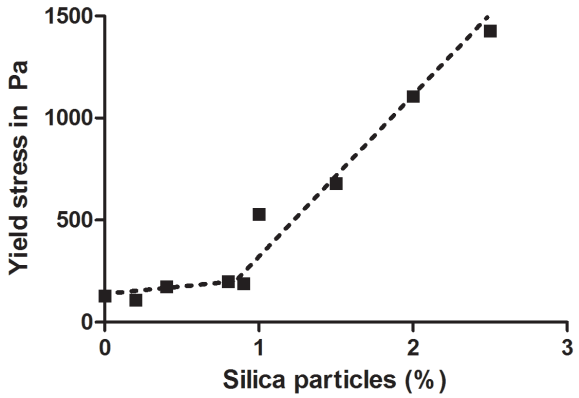

Figure 3. Influence of amount of SiO_2 particles (m/m) added to petrolatum Snowwhite N® on yield stress at 20 °C.

Characteristics of petrolatum components

To study what components in petrolatum are responsible for its crystallinity, X-ray analysis on paraffin wax and microcrystalline wax was conducted. Results are shown in **Figure 4** and show clear peaks in the paraffin wax diffractogram, but not in that of the microcrystalline wax. From this it can be concluded that paraffin wax is the component responsible for crystallinity in petrolatum. Possibly, this is due to the fact that paraffin wax consists of straight chain alkane branches which can potentially organize more easily when compared to branched and cyclic alkanes for microcrystalline wax. This is in line with literature on the influence of branched and cyclic alkanes on the crystallization of n-paraffins (18).

The influence of the viscosity of the oil fraction on the consistency of the composite petrolatum blend was studied by mixing a pre-blend of paraffin wax and microcrystalline wax with oil fractions of different viscosity. The resulting composite petrolatum blend consisted of 40% wax pre-blend and 60% oil. Oil with a viscosity of 5 mPas (at 20 °C) resulted in petrolatum with a yield stress of 162 Pa, whereas oil with a viscosity of 203 mPas (at 20 °C) resulted in petrolatum with a yield stress of 482 Pa. Thus it can be concluded that also oil viscosity may influence yield stress profoundly.

Because of these results, it can be concluded that crystallinity of petrolatum originate from paraffin wax in petrolatum and that also the viscosity of the oil fraction used in petrolatum has a profound impact on product yield stress.

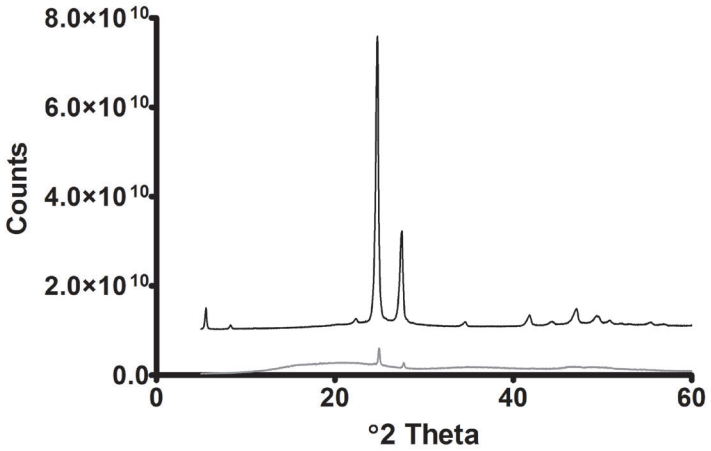

Figure 4. X-ray data of petrolatum waxes, paraffin wax in black and microcrystalline wax shown in grey. The black line has been moved upwards with an increment of $1x10^8$ counts to observe the differences.

Conclusion

The Pharmacopoeia monograph for petrolatum poorly discriminates between grades of petrolatum. As a consequence, Pharmacopoeia quality petrolatum products are not necessarily interchangeable. Petrolatum complying to the Pharmacopoeia can show considerable variations in rheological properties. The results indicate that differences in structure of petrolatum are caused by differences in composition or thermal history. These may influence the amount of crystalline material present in petrolatum and therefore have a profound influence on the rheological properties.

Acknowledgements

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Chapter 2.2

Study of petrolatum structure:

explaining its variable rheological behavior

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Abstract

The rheological properties of petrolatum are dependent on both temperature and thermal history. How this thermal dependency can be explained is unclear. In the past it has been suggested that the structure of petrolatum consists of a three-dimensional crystalline network. This has been established using old microscopic techniques only. Therefore, a study on the microstructure of petrolatum was conducted using rheometry, DSC, pulsed NMR, polarized light microscopy and synchrotron X-ray. The combination of these techniques shows that petrolatum is composed of 21 % solid material at room temperature. This consists of partly crystalline lamellar sheets which are packed in stacks. The occurrence of these lamellar sheets is temperature dependent and the number of lamellar stacks is dependent on thermal history. It was shown that rheological differences in petrolatum can be explained by the number of lamellar stacks present, where more lamellar stacks result in more rigid petrolatum.

Introduction

Petrolatum is one of the most commonly used materials in pharmaceutical and cosmetic ointments and creams. In 2014 approximately 80 million kg of petrolatum was used for pharmaceutical purposes worldwide (1). Therefore, it can be considered a major bulk product for pharmaceutical applications. For petrolatum and other semi solids physical characterization methods are used to describe its consistency. Since it is a viscoelastic material, meaning it combines both viscous and elastic characteristics, its rheological properties are complex. It behaves non-Newtonian and its structural properties are greatly dependent on temperature and applied shear. The rheological properties can be studied in more detail using oscillatory stress testing (2–4). Significant differences in rheological properties exist both between petrolatum grades and different thermal treatments (3).

Understanding what causes differences in rheological properties is essential to optimise manufacturing processes and formulations (5,6). For creams a wide range of studies is described explaining their rheological properties using techniques such as small angle and wide angle X-ray diffraction (SAXS and WAXS, respectively), differential scanning calorimetry (DSC), thermal gravimetrical analysis (TGA) and both electronic and light microscopy (7–12). Since creams contain water, the focus in characterizing these products generally lies on the location of water within the formulation to define whether it is free or bound to structures within the cream. Others focus on the emulsifiers or other distinctive components within the cream. However this does not apply to petrolatum, since it contains no water or emulsifiers but merely consists of alkanes of varying size (13). Therefore, it is more difficult to determine how differences in rheological properties for petrolatum can be explained.

In literature the explanation of differences in rheological properties for semi-solids is often attributed to the gel network paradigm (5). Such a gel network forms a viscoelastic continuous phase in emulsions. For petrolatum the structure is generally described as a two-phase system consisting of a three-dimensional crystalline network consisting of fibre-like crystals that encloses and immobilizes the liquid hydrocarbons (2,4,13–15). No clear evidence exists of this three-dimensional crystalline structure. All cited papers conclude that such a structure may explain the complex rheological behaviour of petrolatum but direct evidence has not yet been described.

Nowadays, new techniques exist to determine structures within materials such as petrolatum. Especially synchrotron X-ray scattering techniques can be powerful in studying structures at detailed level (16). This may finally elucidate the microstructure of petrolatum responsible for its greatly variable rheological properties.

Therefore, we aim to study the nano-, micro- and macrostructure of petrolatum by synchrotron SAXS and WAXS methodologies combined with DSC, pulsed nuclear magnetic resonance (NMR), hot stage polarized light microscopy (HSPLM) and rheometry.

Materials and methods

Materials

Petrolatum (Snowwhite N®, Sonneborn, Amsterdam, The Netherlands) and paraffin oil (Gustav Heess, Leonberg, Germany) were used.

Rheometry

A stress-controlled rheometer (TA instruments HR-2, Etten-Leur, The Netherlands) equipped with a peltier plate and a 40 mm sandblasted parallel plate (TA-instruments plate geometry 40 mm) was used. Approximately 5 gram of petrolatum was placed on the peltier plate before slowly lowering the upper plate to the preset trimming gap of 1050 µm. After trimming excessive petrolatum the geometry gap was set to 1000 µm. The linear viscoelastic region (LVR), meaning the range of stresses within which the structure of the sample is not destroyed (2), was determined using oscillatory stress sweep (OSS) experiments in a wide stress range (1 – 2000 Pa) at 25, 35, 45 and 55 °C. Temperature ramps were conducted within the LVR of 55 ^{o}C at a heating and cooling rate of 5 ^{o}C/min between 25 and 75 ^{o}C. Petrolatum yield stress after slow and fast cooling was determined using a conditioning temperature ramp of 0.1 or 10 °C/min and a OSS similar to the LVR measurements after this temperature ramp. Yield stress was defined as the point where the storage and loss modulus lines cross. Data was analysed using Trios v3.3.0.4055 software.

Differential scanning calorimetry (DSC)

DSC measurements were conducted on a TA Instruments Discovery DSC (TA Instruments, Etten-Leur, The Netherlands). 5-8 mg of petrolatum was placed in DSC hermetic aluminum pans and the sample was conditioned at 10 °C for 10 minutes, next, the sample was heated at 5 °C/min to 70 °C and subsequently cooled to 10 °C at 5 °C/min. Data was analyzed using Trios v3.3.0.4055 software.

Solid fat content (SFC)

Petrolatum was transferred to a glass NMR tube and stored at room temperature for two weeks. Before measurement the sample was conditioned in waterbaths (Lauda Ecoline RE104, New Jersey, USA) at 10, 15, 20, 25, 30, 35 and 40 °C for 30 minutes. Afterwards, pulsed NMR (Bruker Minispec MQ20, Leiderdorp, The Netherlands) was used according to the direct method (17).

Hot stage polarised light microscopy (HSPLM)

A small amount of sample was applied to a glass slide. All samples were analyzed at a 100x magnification on a Nikon Eclipse TE2000-U microscope (Nikon Instruments Europe BV, Amsterdam, The Netherlands). The samples were assessed at different temperatures. The sample was heated to 25°C, then 35°C and 50°C and finally cooled back to 25°C. The estimated average heating rate was 4°C/min, the estimated average cooling rate was 2°C/min. Pictures were analyzed using ImageFocus v 3.0.0.2.

Synchrotron small- and wide-angle X-ray scattering

For small- and wide-angle X-ray scattering (SAXS and WAXS) measurements, a small amount of sample was transferred to 80 mm glass capillaries with a diameter of 1 mm and wall thickness of 0.01 mm (Hilgenberg GmbH, Malsfeld, Germany). The sample was heated homogeneously in an oven to 75°C to remove stresses caused by sample loading. One sample was melted and cooled directly by removing it from the oven at an estimated cooling rate of 10 °C/min. Another sample was allowed to cool to room temperature at a programmed cooling rate of 0.1 °C/min in an oven. Both samples were incubated at room temperature for at least 2 weeks prior to the measurements. After collecting the SAXS/WAXS data for the two samples at room temperature, the fast cooled sample was subject to a thermal treatment protocol as in the rheological measurements.

SAXS and WAXS patterns were obtained at the DUBBLE beamline at the European Synchrotron Radiation Facility in Grenoble (18). The SAXS sample-to-detector distance was kept at 1.5 m and X-rays with a wavelength of 0.1 nm were used to measure the scattering. A Pilatus 1M detector was used to record small-angle (λ) scattering profiles. WAXS profiles were recorded using a Pilatus 300K detector. Background corrections were applied on all azimuthally (integrated across all angles) integrated profiles. Background correction was obtained from scattering recorded from capillaries filled with paraffin oil. In the SAXS profiles the scattering wavevector q is plotted against the corresponding intensity (I). This scattering wavevector can be viewed as the resolution with which the sample is observed and is calculated using Eq. 1, in which λ is the wavelength of the X-rays and θ the angle of the X-rays.

Equation 1: $$q = \frac{4\pi}{\lambda}\sin\frac{\theta}{2}$$

Data analysis

Kratky plot

Before peak analysis, SAXS profiles were multiplied by q^2 to compensate for the inverse square decay (Kratky plot). Such a Kratky plot extracts the slope from the data thereby making the interpretation of the data more reliable since the determination of a peak maximum is more accurate (19).

Porod invariant and peak width σ

Of these Kratky plots, the first peaks were integrated as a measure for the amount of material structured in lamellar sheets. This peak integrant corresponds to the so called Porod invariant (Q) (19). A Gaussian curve was fit to each peak and the peak width σ was taken directly from this fitted Gaussian. This peak width is a measure for the degree of periodic order. This corresponds to the number of lamellar sheets in a stack (19). A high peak width reflects a small degree of periodic order and thus a low number of lamellar sheets in a stack.

Crystal lattice, crystallinity and crystallite size

WAXS peak positions were obtained from the azimuthally integrated WAXS profiles from the peak maxima. From these peak positions the type of crystal lattice was determined. For the amount of crystalline material in the sample, the WAXS profiles were multiplied by q^2 to obtain a Kratky plot. A Gaussian curve was fit to the first peak and a Gaussian was fit to the surrounding background. The ratio of the areas of the Gaussian fit to the first peak and the Gaussian fit to the surrounding background scattering is a measure for the amount of crystalline material in the sample. From the full width at half maximum of the Gaussian fit to the peak we could estimate the size of the crystallites using the Debye-Scherrer equation (Eq. 2) (20). In this equation τ represents the mean size of the crystalline domains, K a dimensionless shape factor with a typical value of 0.9, λ the wavelength of the X-ray, β the line broadening at half the maximum intensity and θ the angle (in degrees).

Equation 2: $$\tau = \frac{K\lambda}{\beta cos\theta}$$

Results and discussion

Macrostructure

Rheometry

A temperature ramp method was conducted on a single petrolatum grade since it is known that thermal history influences the rheological properties of petrolatum (3). In order to define the influence of the nano- and microstructure of petrolatum on rheological properties this was conducted on a single petrolatum grade as a representative model system. Results are shown in **Fig. 1A**.

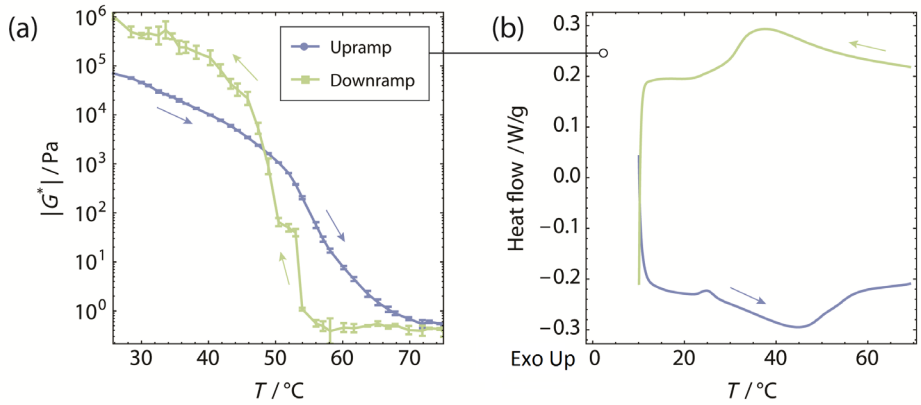

Figure 1. Viscoelastic properties of petrolatum as a function of temperature. The blue line represents the heating curve and the green line the cooling curve. Measurements were conducted in duplicate at heating/ cooling rates of 5 °C/min, SD is shown in error bars (A). Heat flow of petrolatum as a function of temperature at a rate of 5 °C/min. Heating curve is shown in blue and cooling curve is shown in green (B).

Equation 3: $$|G^*| = G' + iG''$$

In **Fig. 1A** on the y-axis a measure for structure of petrolatum is shown, namely the complex modulus |G*|. The |G*| is the complex sum of the storage modulus (G') and the loss modulus (G") (Eq. 3). These two reflect the amount of energy stored in a samples structural interactions when moved, both elastic and viscous. The |G*| can therefore be considered as the total amount of energy involved in the interactions within a sample. A high |G*| means a more rigid structure (21). The |G*| during cooling is lower than the |G*| during heating at temperatures above 45 °C. From approximately 50 °C the cooling |G*| shows a steep increase, becoming higher than the |G*| of the heating curve from approximately 45 °C. Therefore it can be concluded that petrolatum exhibits a different

structure during cooling and heating. The sample becomes more rigid upon cooling than it was before heating. This means that thermal history greatly influences petrolatum structure.

Differential scanning calorimetry (DSC)

The macrostructure of petrolatum can be further evaluated using DSC in which structural characteristics such as melting can be determined (22). Results are shown in **Fig. 1B**.

Fig. 1B shows that during heating endothermic reactions occur while during cooling exothermic reactions occur. The shape of the peaks during heating and cooling appear to be slightly different. The enthalpies (±95% CI) can be calculated by integrating the peaks in the thermogram. During heating the enthalpy is 18.9 (±1.26) J/g and during cooling 23.1 (±1.04) J/g. During cooling significantly ($p<0.05$) more energy is released in forming petrolatum structure than is needed to melt its structure. This is in line with the rheometry data suggesting that during cooling a different structure is formed than the structure that melts during heating (**Fig. 1A**).

Solid fat content

The solid fat content was determined for petrolatum using pulsed NMR (**Table 1**). With this method, the amount of solid material can be determined in a sample. The molecules in a solid have different resonance properties than those in liquids. Using NMR these two can be distinguished to quantify their relative proportions (17).

Table 1. Solid fat content of petrolatum determined by pulsed NMR.

Temperature in °C	Solid fat content (in % of total mass)
10	24.4
15	23.0
20	21.9
25	20.9
30	19.2
35	16.7
40	14.1

In **Table 1** it can be seen that at lower temperatures petrolatum contains relatively more solid material. At 25 °C this amount is 20.9%. Such a percentage is in line with literature on the composition of petrolatum in general, stating that it consists of only 7-13% high molecular weight paraffins, 30-45% smaller paraffins and 48-60% small paraffins (13,23).

When we assume that the length of the molecules determines whether it is solid or liquid at a certain temperature, it seems logical that all high molecular weight and part of the smaller paraffins are solid at 25 °C.

Microstructure

Synchrotron small angle X-ray diffraction (SAXS)

To see how the microstructure may influence the rheological properties the structure was studied using synchrotron SAXS measurements. A capillary was filled with petrolatum and exposed to the same temperature protocol as the rheometry measurements of **Fig. 1A**. In **Fig. 2** the results of the SAXS measurements are shown, intensity (I) times q^2 is plotted against the scattering wavevector (q in nm, Eq. 1).

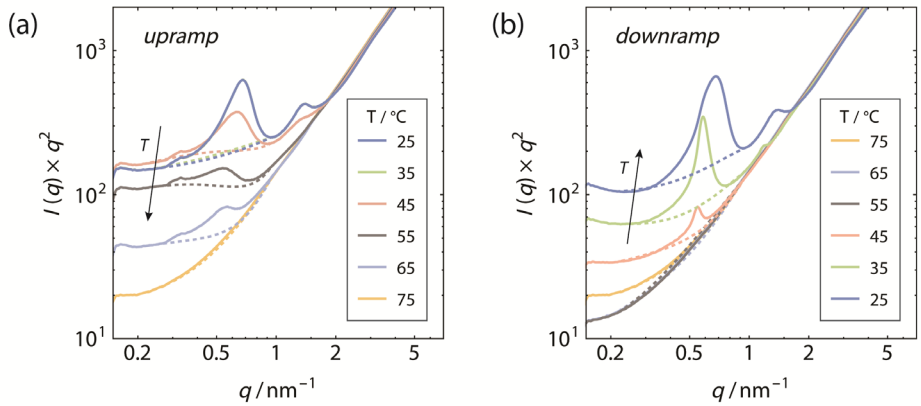

Figure 2. Kratky representations of the heating (A) and cooling (B) synchrotron small angle X-ray diffraction (SAXS) data. Peak integration lines are shown (dashed).

Fig. 2A and **B** show several scattering profiles for different measurement temperatures. In some of these, especially at lower temperatures, peaks can be observed. These occur around 0.6 nm^{-1} and repeat at around 1.2 nm^{-1}. The peaks start to disappear during heating and are absent above 65 °C. When the sample is cooled (**Fig. 2B**) the peaks start to reappear at temperatures lower than 55 °C and show a different, sharper shape than during heating, meaning that SAXS patterns are different during heating and cooling for petrolatum.

The peak pattern in the SAXS measurements indicates the presence of structures in the sample. Based on the fact that the peaks repeat it appears that the structures are periodic. For SAXS measurements it is known that this is a signature of stacks of lamellar

sheets with periodicity in one direction (24). The data follows a horizontal trend at small angles. If the structures that are present were smaller than ~1 µm, the scattering intensity would not have been horizontal within the *q*-range probed in the experiment. Therefore, it seems likely that the length of the lamellar sheets in petrolatum is at least in the micrometer range.

In **Fig. 3A** the Porod invariant, obtained from the Kratky-representation, was plotted against the temperature. The peak width σ is plotted against temperature in **Fig. 3B**.

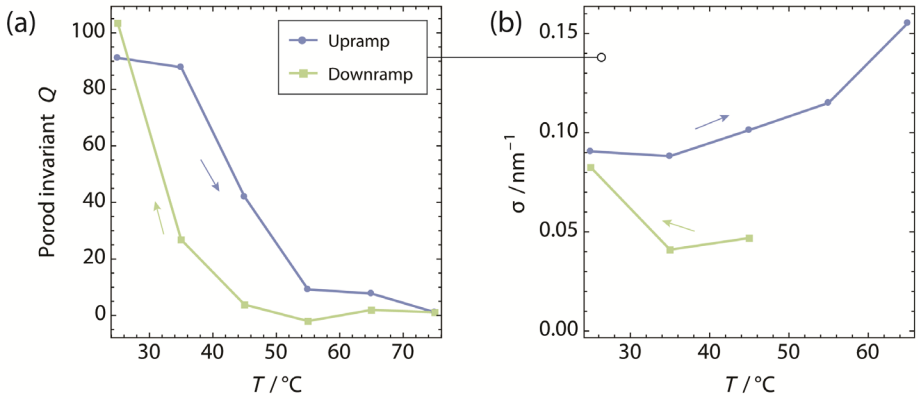

Figure 3. Porod invariant (Q) integrated from Figure 3 plotted against the temperature (A). The peak width calculated by fitting a Gaussian curve, expressed as σ/nm^{-1} plotted against temperature for the Kratky plots (Fig. 3) (B).

Fig. 3A shows that for the cooled sample the Porod invariant is lower compared to the heating curve except for the data point at 25 °C. This means that at 25 °C more material is structured in lamellar sheets after cooling than before heating. During cooling however less material is structured in lamellar sheets than during heating at temperatures higher than 25 °C. In **Fig. 3B** the peak width, which corresponds to the number of lamellar sheets in a stack (19), is plotted against temperature. What can be clearly seen is that during heating the peak width increases whilst during cooling the peak width is smaller. The structure of petrolatum can be interpreted as having better defined lamellar stacks during cooling than during heating. At room temperature the standard deviations are similar for both the starting material and cooled sample (**Fig. 3B**).

In summary it can now be said that in petrolatum lamellar sheets exist which are ordered in stacks. Their presence and degree of ordering in stacks is temperature dependent.

Influence of cooling rate on petrolatum structure

From the results discussed in the previous sections we can conclude that the lamellar structure of petrolatum is different directly after cooling compared to the starting material. To study whether this difference persists after storage at room temperature, two samples with different thermal history were created (analogues to (3)). One was cooled fast (10 °C/min) and one slow (0.1 °C/min) to room temperature and stored for two weeks. Following this storage period, synchrotron SAXS profiles were obtained at 25 °C, as described above.

For these measurements Kratky presentations were made to estimate the Porod invariant and the peak width. All these are shown in **Fig. 4**.

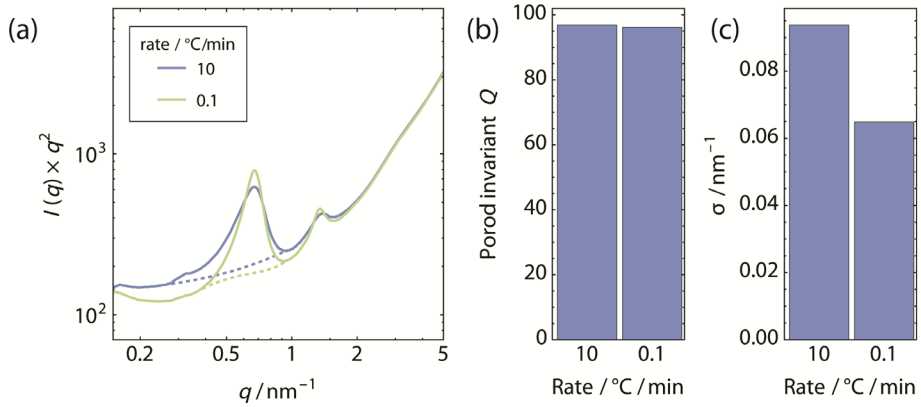

Figure 4. Synchrotron small angle X-ray diffraction (SAXS) measurements of petrolatum stored for two weeks at room temperature after different thermal treatments in which samples were cooled to room temperatures at different rates, 0.1 and 10 °C/min. Fig. 4A represents the Kratky representation of the synchrotron measurements. The area under the first peak of Fig. 4A is plotted in Fig. 4B as Porod invariant *Q* and the peak width represented as σ/nm^{-1} by fitting a Gaussian curve to the peak in Fig. 4C.

In **Fig. 4A** it can be seen that for the slowly cooled sample (0.1 °C/min) sharper peaks are shown. The peak pattern is similar to the pattern shown for the heating and cooling ramp of **Fig. 2**. Thus the lamellar sheets that arise during the cooling of petrolatum are still present after storage at room temperature for two weeks. Interestingly, no clear difference in Porod invariant for the two thermal treatments exists. Thus similar amounts of material are structured in lamellar sheets for the two samples. The peak width (**Fig. 4C**) on the contrary, shows a clear difference. The faster cooled sample consists of lamellar sheets that are less ordered or contain less layers than those in the slowly cooled sample. As has been described before, petrolatum exhibits a more rigid structure

after fast cooling compared to slow cooling (3). To illustrate this the yield stress, which is a measure for the rigidity of petrolatum structure, was measured. For a fast cooled (10 °C/min) sample the yield stress is 1721 Pa and for a slowly cooled (0.1 °C/min) sample 466 Pa. This indicates that not only the number of lamellar sheets but also the degree of ordering of the lamellar stacks influences petrolatum rheology.

Hot stage polarized light microscopy (HSPLM)

To visualise the lamellar stacks in petrolatum HSPLM was performed. Results are shown in **Fig. 5**.

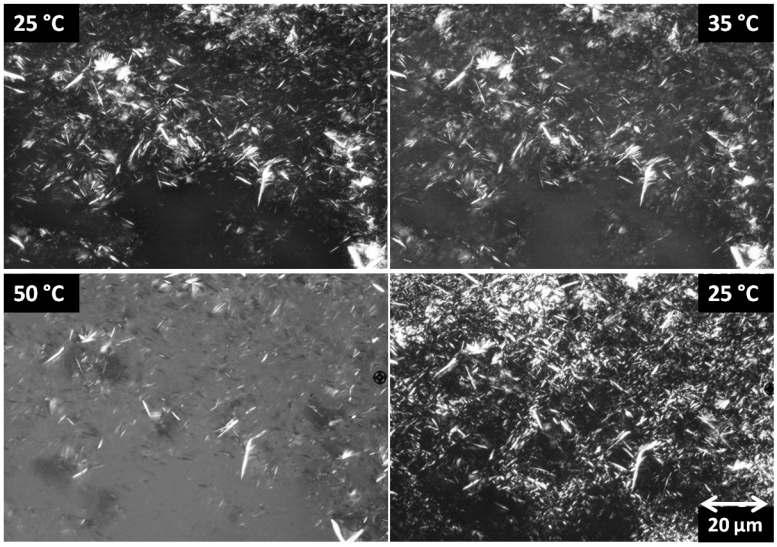

Figure 5. Hot stage polarised light microscopy (HSPLM) pictures of petrolatum at different temperatures. Magnification was 100x, estimated heating rate was 4 °C/min and cooling rate 2 °C/min. Order of the pictures is from left to right.

What can be seen in **Fig. 5** is that needle like structures are present in petrolatum of approximately 5-30 µm in size. The amount of these structures is dependent on temperature showing that at 50 °C almost all is melted. Using polarised light microscopy materials can be visualised because of birefringence, meaning a refractive index depending on the polarization of light. Lamellar stacks have a different density compared to the amorphous surroundings and can therefore be visualised using HSPLM. As a result, the observed needle like structures most likely correspond to the lamellar stacks detected in the SAXS measurements. Similar microscopic techniques have previously been used for petrolatum, the observed structures were linked to a three-

dimensional crystalline network that encloses and immobilizes the liquid hydrocarbons (13,25,26). This however is impossible to conclude on the basis of merely microscopic techniques. Furthermore, the suggestion of crystallinity can be better estimated using X-ray diffraction techniques.

Nanostructure

Synchrotron wide angle X-ray scattering (WAXS)

Using synchrotron WAXS the crystallinity of petrolatum can be determined. Experiments were conducted on the same samples and conditions as the SAXS measurements and results are shown in **Fig. 6**.

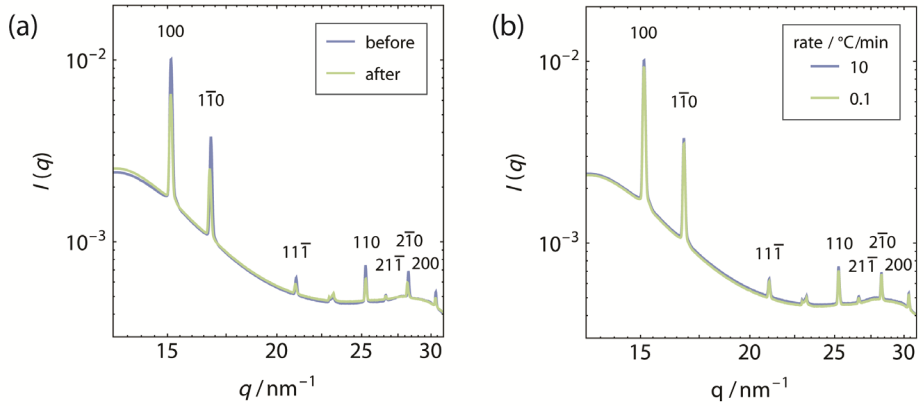

Figure 6. Synchrotron wide angle X-ray scattering (WAXS) experiments on petrolatum before and after heating and cooling (A) and petrolatum stored for two weeks at room temperature after different thermal treatments in which samples were cooled to room temperatures at different rates, 0.1 and 10 °C/min (B).

In **Fig. 6A** several sharp peaks can be seen for both samples, the peaks show similar sharpness and positions for both samples. The sample shows lower peaks after cooling compared to the sample starting material. The peak area is a measure for the amount of crystalline material present. Lower peaks after cooling consequently suggest that the appearance of crystalline order on the nanoscale is lower compared to the starting material. For the samples with different thermal treatments the same peaks can be seen, no clear difference exists between the slowly and fast cooled sample. Therefore, it can be concluded that crystal formation is slow and continuous during storage.

The peak positions give information on the crystal lattice and an interpretation of the crystal type is shown in **Fig. 7A**. The peaks span a rhombohedral lattice with an angle

of 113° and a lattice parameter of around 0.4 nm. The larger peaks can all be ascribed to this lattice. The smaller peaks (111 and 211) can be ascribed to a three dimensional lattice. These structures do not disappear upon heating in contrast to the rhombohedral structures (data not shown). Probably, the small peaks are other structures than the alkanes of petrolatum. Potentially impurities.

Application of the Debye-Scherrer equation (Eq. 2) to the width of the first WAXS peak leads to an approximate crystal domain size of up to 50 nm, comparable to what was found in a previous study (3). The mass fraction of crystalline material was found to be in the order of ~8%. Using pulsed NMR it was shown that at 25 ^{o}C 20.9% of petrolatum is solid. If we assume that all solid material in petrolatum is composed of lamellar sheets, ~40% of the lamellar sheets consists of crystalline domains. The percentage of ~8% crystalline material is higher compared to the previously reported value of 1.5% (3). This difference may be caused by differences in integration method of the WAXS data and/or due to the lower accuracy of the powder X-ray diffractometer used in (3) compared to the synchrotron WAXS used in the current study.

Synchrotron small angle X-ray scattering (SAXS)

Using synchrotron SAXS not only the length of the lamellar sheets can be estimated but also the thickness of the lamellar sheets can be determined using Eq. 4.

Equation 4: $$d = \frac{2\pi}{q}$$

The periodicity of the lamellar sheets is approximately 10 nm, meaning that lamellar sheets repeat every 10 nm and are most likely separated by lower density liquid. The individual thicknesses of the sheets (a) and lower density fluid (b) are uncertain, however the sum of the two does not exceed 10 nm, as is represented in the **Fig. 7B**.

Concluding remarks on petrolatum structure

Fig. 7 represents a graphical summary of our findings on petrolatum nano-, micro and macrostructure. Clearly, on the smallest scale, a fraction of molecules in petrolatum is ordered in a rhombohedrical crystal lattice (**Fig. 7A**), forming crystalline domains of approximately 50 nm (**Fig. 7B**). The crystalline domains are part of lamellar sheets that repeat every 10 nm in one direction. The formation of the crystals is slow during cooling compared to the lamellar sheets, which form relatively fast. Thermal history primarily influences the degree of ordering of the lamellar sheets in stacks (**Fig. 7C**). These lamellar stacks make up the macrostructure of petrolatum. The evident influence of the lamellar stacks on the macrostructure of petrolatum is shown in the significant alterations in rheological properties depending on the number of lamellar sheets and lamellar stacks present. The amount of crystalline domains in petrolatum on the other hand does not seem to influence its rheological properties.

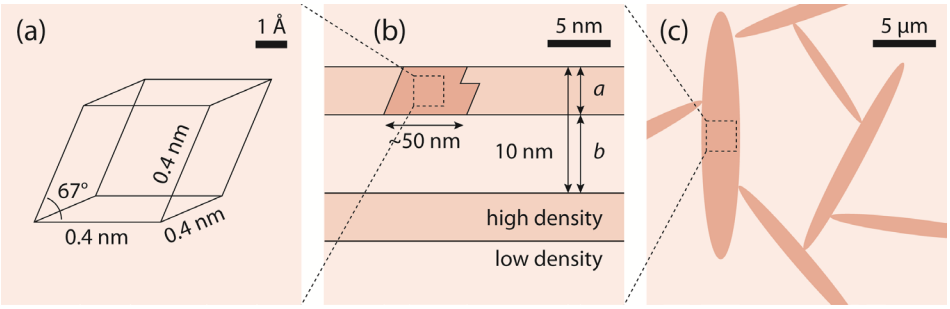

Figure 7. Schematic overview of the findings on the structure of petrolatum. The Ångstrom scale rhombohedral lattice structure of the crystalline fraction in petrolatum (A), the lamellar structure on a nanoscale (B) and the structure on micrometer scale of interacting lamellar stacks (C) is shown.

Petrolatum is a viscoelastic material and it is now shown that the presence of lamellar stacks can be linked to petrolatum rheological properties. As such, light can finally be shed on the microstructure paradigm. Previously, a fibre-like crystalline network was used to explain the rheological properties of petrolatum (2,4,13–15). In this study we provide compelling evidence that the fibre-like structures are actually partly crystalline stacks of lamellar sheets that trap the liquid fraction of petrolatum. Therefore, a shift in paradigm from a fibre-like crystalline network to a network of partly crystalline lamellar stacks that interact when moved is proposed.

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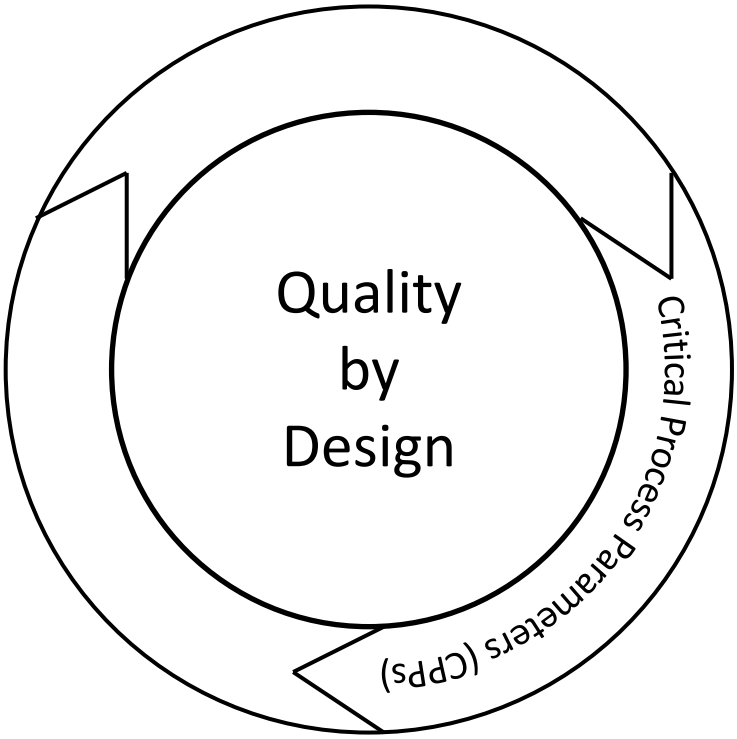
Chapter 3

Critical Process Parameters (CPPs)



Chapter 3.1

The influence of cetomacrogol ointment processing on structure: a definitive screening design

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Abstract

Batch-to-batch variability is a challenge for the industrial scale production of ointments. Therefore, the current investigation focussed on identifying and understanding critical process parameters (CPPs) for cetomacrogol ointment. This was evaluated using a Definitive Screening Design (DSD) approach in which fourteen batches were produced under predefined and controlled conditions using the following variables: addition of SiO_2 nanoparticles, mixing speed, cooling rate, heating temperature, container filling temperature and isothermal mixing at the filling temperature.

Ointment structure was evaluated using a number of rheological parameters. One of these parameters, yield stress was found to be strongly influenced by filling temperature and mixing speed ($P = 0.0065$ and $P = 0.0013$ respectively). Both significantly affect ointment structure and they also have a significant interaction ($p<0.05$). Understanding the ointment production process can help in defining a processing window to produce ointment of constant quality.

Introduction

Ointments are widely used in the therapy of various skin diseases. When ointments are produced on an industrial scale, unexplainable batch-to-batch variation in ointment structure may occur (1). An example of a product that is prone to such batch-to-batch variation is the water free cetomacrogol ointment. Examples of reported complaints concerning cetomacrogol ointment involve the presence of agglomerates and the occurrence of a highly viscous product that cannot be easily removed from a tube or container (2). The variation between batches remains high due to a lack of understanding and control of parameters in the production process. This leads to rejection of batches and decreases the productivity of pharmaceutical companies.

Until now, relatively few articles have described the influence of formulation and process parameters on ointment structure (3–5). Furthermore, they report conflicting results. For ophthalmic ointments, formulation and process factors were studied for product quality and *in vitro* performance. Processing was found to have a minor impact on the particular ophthalmic ointment structure (3). On the other hand, a study on creams concluded that processing may impact product structure substantially (4).

This highlights the importance of a systematic study on the influence of the manufacturing process on consistency and physical stability of topical products (1).

Structural properties of ointment-like products are generally characterized using rheological testing. These tests provide information that can be linked to results of sensory test panels and are therefore able to describe sensory properties well (6,7). In order to conduct a systematic study on the influence of formulation and processing variables on ointment structure a Quality by Design (QbD) approach seems preferable. QbD is a systematic tool to study the influence of formulation and process variables. The influence of process variables on product quality can be identified using a design of experiments (DoE) approach. With a screening design such as a fractional factorial design, the criticality of process variables can be estimated by determining the critical process parameters (CPPs) (5).

These screening designs are available in many different varieties. Classical designs generally study variables at two levels and as such can say nothing about whether a nonlinear relationship between the two tested levels exists. This has led to the recent

development of a new type of screening design has been developed that seems more powerful. In this Definitive Screening Design (DSD) variables are studied at three levels rather than two levels, and it is possible to examine possible interactions between variables (8).

In short, given the current lack of ointment process understanding, the aim of this study is to identify critical process parameters and their influence on ointment critical quality attributes. This will be done by using a Definitive Screening Design approach.

Materials and methods

Materials

All materials complied with the quality requirements of the European Pharmacopoeia (Ph. Eur. 2016). White petrolatum Snowwhite N® (Sonneborn international, Amsterdam, the Netherlands), cetomacrogolwax Galenol® 1618 AE (Sasol GmbH, Brunsbüttel, Germany), isopropylmyristate Kollicream® IPM (BASF Personal Care and Nutrition GmbH, Düsseldorf, Germany), paraffin 110-230 mPa.s (Gustav Heess GmbH, Stuttgart, Germany) and SiO_2 particles Aerosil® 200 vv Pharma (Evonik, Paris, France).

Batch production

The batches (0.5 kg) were produced under controlled conditions in a 4M8-Trix homogenizer (ProCePt, Belgium). Cooling rate was defined in the range from 45 °C to filling temperature. In this range the solidification of the ointment occurs. A cooling rate of 0.15 °C/min was achieved by cooling down the mass without water. Cooling rates of 0.575 and 1.00 °C/min were achieved by cooling the wall of the vessel with water (low versus high flow). After production samples were stored for two weeks at room temperature. After this initial period of storage, samples were analysed. Prior to analysis, the samples were preconditioned for at least 1 hour in a peltier-cooled incubator (Memmert IPP30) at 20 °C. Production and analysis of the sequence was randomized.

Rheological characterization

The stress-controlled rheometer (TA Instruments Discovery HR-2, Etten-Leur, The Netherlands) used was equipped with a step-peltier stage (20 °C) and a 40 mm sandblasted parallel plate (TA-instruments Cone plate geometry 40 mm, Etten-Leur, The Netherlands). After approximately 5 gram of ointment sample was placed on the peltier plate, the upper plate was slowly lowered to the preset trimming gap of 1050 µm. After trimming excessive ointment the geometry gap was set at 1000 µm. Before analysis, samples were equilibrated for 5 minutes at 20 °C. All rheological studies were performed in triplicate and results were expressed as mean ± relative standard deviation (RSD %). To characterize the rheological characteristics, the following procedures were performed in sequence on each sample:

- Oscillatory stress sweep: a logarithmic stress sweep at a frequency of 1 Hz was conducted within the range of 10 to 2000 Pa. The point of intersection with the G ‘and G’’ was defined as yield stress.

- Flow ramp: a linear flow ramp from 0.1 to final 200 s^{-1} was measured for 120 seconds. Outcomes zero shear viscosity and viscosity were determined using this measurement.
- Axial compressibility: 5 gram ointment was compressed to a distance of 1000 µm (distance upper and lower plate), gap speed 10.0 µm.s^{-1} and angular velocity 0.0 rad.s^{-1}. The total axial force was measured at 1000 µm.
- Axial tension: after reaching 1000 µm, the direction of the upper plate was moved in the opposite direction, gap speed 10.0 µm.s^{-1}.

Critical quality attributes (CQAs)

The following critical quality attributes (CQAs) were studied: yield stress, RSD in yield stress, linear viscoelastic region (LVR)-height, viscosity, zero shear viscosity, axial compression and axial tension. Yield stress and LVR-height are measures for ointment spreadability. Zero shear viscosity, axial compression and axial tension are parameters to evaluate ointment structure in its container. Zero shear viscosity is the viscosity of the ointment when no shear is applied. Axial compression is the amount of force that is needed to compress a certain volume of ointment and axial tension is a measure for the stickiness of ointment to the geometry.

X-Ray diffractometry

Room temperature XRD measurements were carried out on a Bruker-AXS D8 Advance powder X-ray diffractometer, in Bragg-Brentano mode, equipped with automatic divergence slit and a PSD Våntec-1 detector. The radiation used was Cobalt $Ka_{1,2}$, l = 1.79026 Å, operated at 30 kV. Crystallite size was calculated using the Scherrer equation (Equation 1) by using a K-value of 0.89 and calculating the β value by measuring the width of the peak at half the maximum intensity and substracting the instrumental line broadening.

Equation 1: $$\tau = \frac{K\lambda}{\beta cos\theta}$$

Crystallinity was determined using the ratio between background and peak area, calculated with the XRD analysis program DiffracEVA (Bruker, The Netherlands).

Design of experiments

Each batch consisted of: white petrolatum (38.25 %), paraffin 110-230 mPa.s (21.25 %), cetomacrogolwax (25.5 %) and isopropylmyristate (15%). A DoE with 14 runs (**Table 1**), made of a mixture design with 5 continuous variables at 3 levels combined with 1 discrete

factor at 2 levels, was used during this study. Six parameters were varied: maximum product temperature: ProdT (60.0 – 80.0 ˚C), SiO_2 particles: SilP (0.0 – 0.10%), mixing rate: MixR (10-100 rpm), cooling rate: CoolR (0.15-1.0 °C.min^{-1}), exit temperature: ExitT (30.0 – 37.0 °C) and 10 minutes mixing in the last step before filling: MixF (Yes/No) (**Table 1**). The resulting products were analyzed for a number of CQAs; these are specified in the paragraph critical quality attributes. After model analysis optimal formulation and process variables were identified; this model was subsequently validated in triplicate by producing at optimal settings and measuring the CQAs.

Table 1. Detailed experimental conditions for the DoE. ProdT = Product temperature, MixS = Mixing speed, CoolR = Cooling rate, ExitT = Exit temperature and MixF = 10 minutes isothermal mixing before filling.

#	ProdT (°C)	Addition SiO_2 (%)	MixS (rpm)	CoolR (°C/min)	ExitT (°C)	MixF
1	70	0.05	55	0.575	33.5	YES
2	80	0.10	100	0.15	33.5	NO
3	80	0.05	10	1.0	37	NO
4	60	0.10	55	1,0	30	NO
5	70	0.05	55	0.575	33.5	NO
6	60	0.05	100	0.15	30	YES
7	80	0.10	10	0.575	30	YES
8	80	0.00	55	0.15	37	YES
9	60	0.00	10	1.0	33.5	YES
10	70	0.00	10	0.15	30	NO
11	60	0.10	10	0.15	37	NO
12	80	0.00	100	1.0	30	YES
13	60	0.00	100	0.575	37	NO
14	70	0.10	100	1.0	37	YES

CQA target windows

Critical quality attributes were established beforehand based on rheological properties commonly described in literature and on results of sensory testing by an internal test panel (n=10). In the latter case, panel members were asked to evaluate different batches of cetomacrogol ointment and determine what they felt appropriate for patient use. Based on the results the following targets were set: yield stress (500-800 Pa), RSD yield stress, expressed as Ln RSD (minimization), LVR height (400.000 – 600.000 Pa), zero shear viscosity (500 – 750 Pa.s), viscosity 20 °C (6.0 – 9.0 Pa.s), axial compressibility (10-25 N) and axial tension (10 – 30 N).

Statistical analysis

Analysis was conducted according to the methodology for definitive screening designs (8) by an independent statistical expert (Stanwick, Merelbeke, Belgium). Software from SAS, JMP-12 was used. For every CQA, a statistical analysis was conducted and finalized by analysing the residuals plot for potential outliers and for confounding using the VIF function (VIF < 5). This analysis results in a model function by which values for CQAs can be predicted based on input variable settings.

For every single CQA the analysis was stored and subsequently all CQAs were analyzed together in order to optimize all input variables for all CQAs. This optimization produced desirability graphs that showed whether chosen settings for a variable could produce ointment on target, a value of 1.0 means completely on target and 0.0 completely off target.

The outcomes of the statistical analysis for single CQAs or for all CQAs are referred to as "model".

Results and discussion

The following outputs were evaluated in the current study namely yield stress, homogeneity (in %RSD), LVR-height, viscosity, zero shear viscosity, axial compression and axial tension. On all outcomes, stepwise regression analysis was conducted to design a model that estimates the influence of formulation and process variables on CQAs. The model for yield stress is described below as an example. Subsequently, all CQAs were analyzed together to design a final model for the optimization of cetomacrogol ointment production process.

Model for yield stress

In the current study, yield stress (at 20 °C) of the products was found to lie between 272 and 1309 Pa (**Figure 1**).

As can be seen, only 5 of the 14 produced batches are within the specification for yield stress. Yield stress was shown to be an important parameter for pharmaceutical and cosmetic materials when considering storage stability and sensory features such as spreadability (9). Based on sensory testing by an internal test panel (N=10), yield stress values may be roughly categorized as follows: yield stresses < 400 Pa are more cream-like and ointments with yield stress > 800 Pa are too thick to be removed from a tube.

Therefore the observed differences in yield stresses for the different tested products (272 – 1309 Pa) will have a substantial impact. To assess the impact of formulation and process variables on ointment yield stress, a non-linear statistical model was created; a summary is shown in **Figure 2**.

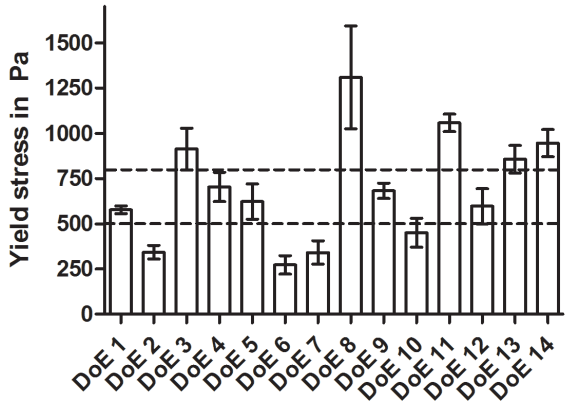

Figure 1. Yield stress results for DoE ointment batches measured at 20 °C, CQA window is defined in the range of 500-800 Pa (shown in dashed lines). Yield stress is expressed as mean ±SD.

Exit temperature, mixing rate and addition of SiO_2 particles all showed a significant effect (p-values: 0.0065, 0.0013 and 0.0073 respectively). Furthermore, mixing rate and exit temperature showed a statistically significant interaction (p-value 0.0116). The statistical function for this model given by Equation 2 can be used to estimate the yield stress with a certainty shown in the ANOVA of F = 50.2 and a probability of p = 0.0009. This means that statistical significant differences have been found in the analyzed data. Furthermore, the model is able to explain 97.2 % of variation in the yield with a R^2adjusted = 0.972 and no lack of fit p > 0.05. Therefore, this model can be considered as a reliable way to describe cetomacrogol ointment yield stress.

Equation 2:

$$Yield\ stress\ (Pa) = -1909{,}074 - 1155{,}90 * SiO_2 + (SiO_2 * SiO_2 * 75815{,}70) - 0{,}96 * \mathrm{MixR} + (\mathrm{MixR} * \mathrm{MixR} * -0{,}13) + 91{,}78 * \mathrm{CoolR} + 77{,}06 * \mathrm{ExitT} + (\mathrm{ExitT} * \mathrm{ExitT} * 14{,}74) + (\mathrm{MixR} * \mathrm{CoolR} * 1{,}86) + (\mathrm{MixR} * \mathrm{ExitT} * -0{,}57)$$

For all six other CQAs (overview shown in **Figure 2**) similar statistical functions were calculated using the same approach and subsequently analysed together to design a model for all CQAs.

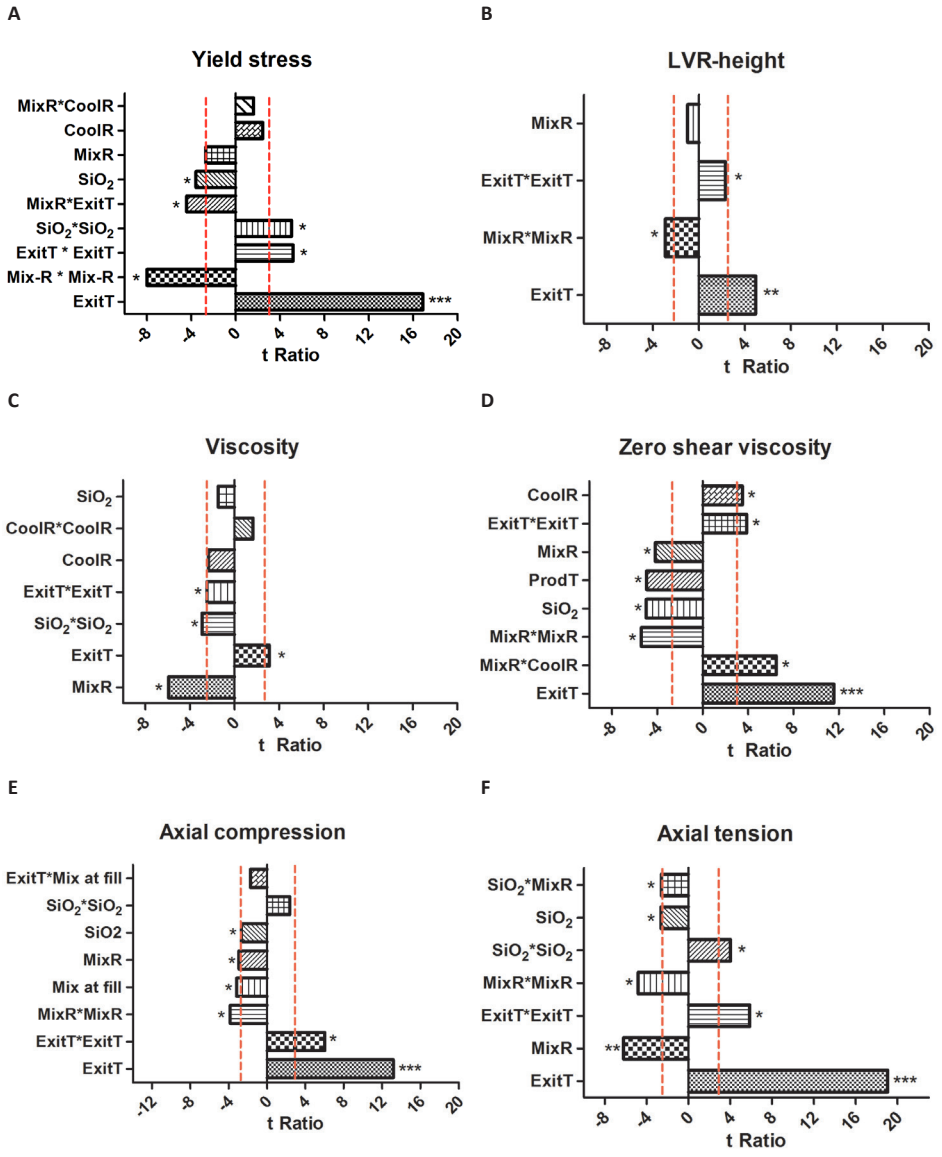

Figure 2. Sorted parameter estimates based on t-ratio for various formulation and process parameters. * p-value<0,05, ** p-value<0,001, *** p-value<0,0001

Optimizing formulation and process variables for all CQAs

The overview of the effects of the five process variables is shown in **Figure 3**; all significant effects are marked with an asterisk (*) and acceptable ranges for the CQAs are shown in grey bars.

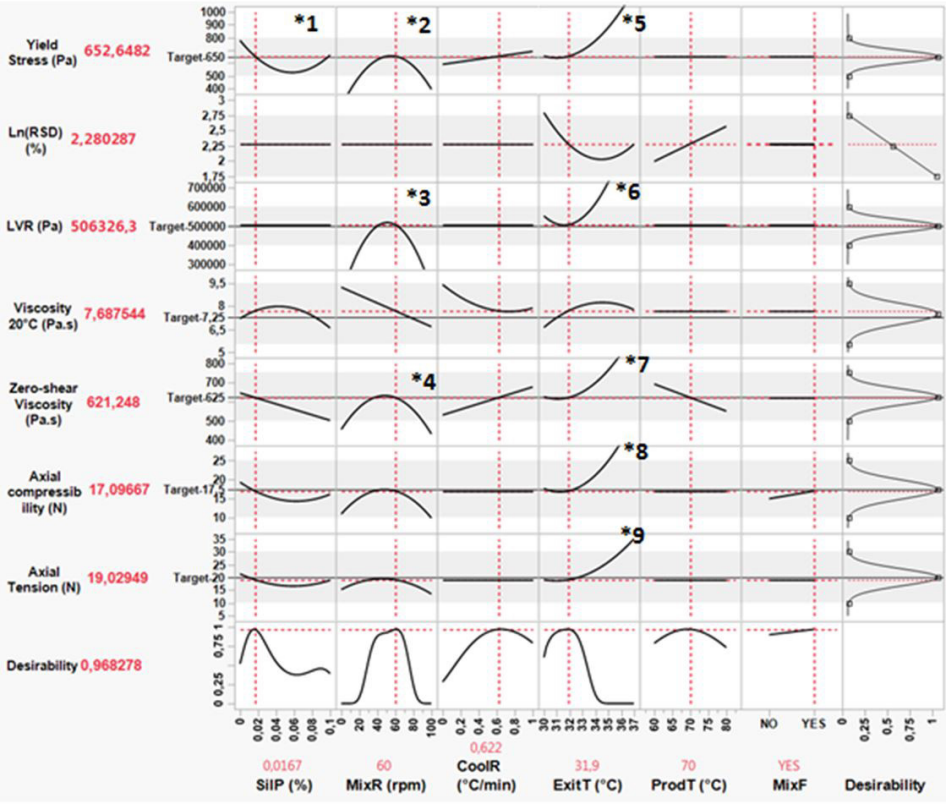

Figure 3. Summary of all outcomes from DoE study, significant results *. Acceptable target range shown in grey. 1) p = 0.02307 2) p = 0.0013 3) P= 0.01757 4) P=0.00292 5) P=0.0065 6) P= 0.04679 7) P= 0.01159 8) P= 0.00180 9) P=0.00109. SilP = silica nano particles; MixR= Mixing rate; CoolR = Cooling rate; ExitT = Exit temperature; ProdT = Product temperature; MixF = 10 minutes isothermal mixing.

In **Figure 3**, it can be seen that mainly mixing rate and exit temperature had an important and statistical significant effect on several of the CQAs, namely yield stress, LVR-height, zero shear viscosity, axial compression and axial tension. The addition of SiO_2 particles (within the tested range of 0.0 % - 0.1%) also has statistical significant effects, however this effect does not influence any CQA enough for it to be out of specification. Therefore, addition of SiO_2 particles to the formulation is not considered critical. Interestingly, research from the Dutch pharmaceutical association (FNA) has shown that the addition

of 0.1% of SiO_2 particles is critical for large scale production (2). As has been shown in this study, apparently this is not the case for at least small scale production.

The desirability graphs for all CQAs in the bottom of **Figure 3** show the optimal settings for the process variables. From these graphs, it can be concluded that mixing rate is critical and that the optimal rate lies in the middle; 60 rpm. Furthermore, exit temperature, which is the temperature at which containers are filled, has a significant influence and should therefore be precisely controlled. Preferably, temperature for filling cetomacrogol ointment containers should be 31.9 °C. Furthermore, it can be concluded that for any tested cooling rates between 0.15 – 1.0 °C/min, all CQAs are within specification. Heating temperature (ProdT) has no significant impact on any of the CQAs, nor did mixing at filling temperature for 10 minutes.

To estimate whether this model is able to predict the influence of all studied parameters on cetomacrogol ointment structure, verification was conducted in triplicate. For this verification, ointment was produced at the optimal settings (shown at the bottom of **Figure 3**) and all in the DoE studied CQAs were analysed. Results (see **Table 2**) show that the RSD between the three batches was between 1.0 and 11.6%. The deviation of the average outcome from the target value is relatively high for LVR-height and axial tension (8.6% and 15.3% respectively). However, deviations in rheological analyses are prone to be high: from 10 to 20% depending on the used equipment and methods. Therefore, this variation between measurements can be considered to be normal; and additionally, important parameters such as yield stress show values close to the target setting (-2.8%). Thus, all three individual batches were within specification and based on these results, the model can be considered valid.

Table 2. Summary of verification batches produced at optimal settings: ProdT 70 ^oC, ExitT 31.5 ^oC, MixS 50 rpm, SiO_2 0.0167%, CoolR 0.6 ^oC/min.

Output parameter	Target	RSD between validation batches (%)	Deviation of average from target (%)
Average Yield stress (Pa)	653	3.6	-2.8
Ln (RSD)	2.28	11.6	2.3
Average LVR height (Pa)	506326	8.1	8.6
Average viscosity 20 °C (Pa.s)	7.7	4.2	-2.0
Average zero shear viscosity (Pa.s)	621	1.0	3.1
Average axial compressability (N)	17.1	8.9	7.8
Average axial tension (N)	19.0	6.1	15.3

Understanding mechanisms behind significant parameters

In order to understand why mixing rate and exit temperature have a significant impact on ointment structure, further experiments were conducted.

To study the effect of mixing rate in more detail, fresh ointment batches were produced, similar to the DoE batches, with mixing rate as the only variable. Batches used in the DoE were not used, since in this design no batches were produced with only this process variable. Data are shown in **Figure 4**. It can be seen that the pattern is similar to the pattern found in the model. Since it is known that crystallinity may influence structure, X-ray analysis was conducted on these samples (10).

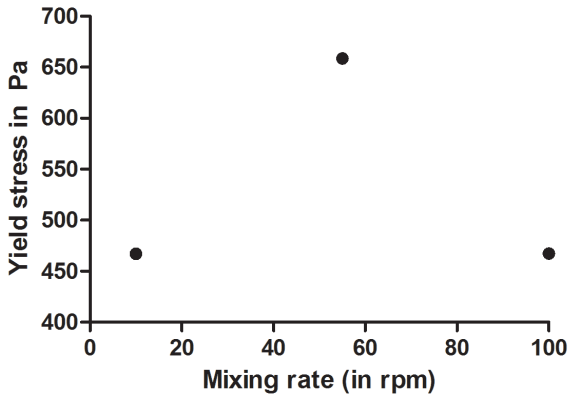

Figure 4. Influence of mixing rate on yield stress. Processing conditions: ProdT 70 °C, ExitT 31.5 °C, MixS 50 rpm, SiO_2 0.014 %, CoolR 0.6 °C/min.

The parabolic relationship for yield stress dependency on mixing rate can possibly be a consequence of a higher percentage of crystalline material in the 55 rpm sample compared to the 10 and 100 rpm samples (5.6% compared to 4.7% and 4.3%). These small differences in crystallinity may contribute to a significant difference in yield stress, because the crystallites are very small (approximately 20-50 nm). Crystallite size was determined using X-ray diffractometry, a convenient method for determining the mean size of nanosized crystallites and sample preparation that is less destructive (11). As was shown in (12) particles of nanosize have a major effect on ointment yield stress. Since SiO_2 particles of approximately 15 nm were used in the current study it was expected that these would also have a major effect on ointment yield stress. However as **Figure 5** shows, an apparent threshold for the ability of SiO_2 to increase the yield stress in cetomacrogol ointment lies above the concentrations used in the DoE. Therefore, it

can be understood why SiO_2 did not have a significant impact on the consistency of cetomacrogol ointment. Similar phenomena have been described for other materials such as polymer composites, polyhydroxyalkanoates and inulin (10,13,14).

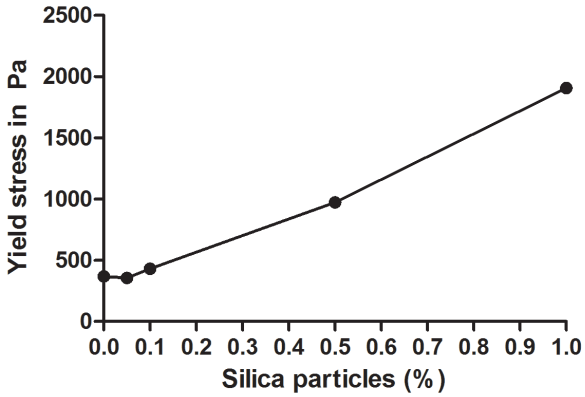

Figure 5. Effect of SiO_2 particles on yield stress of cetomacrogol ointment. Processing conditions: ProdT 70 C, MixS 55 rpm, CoolR 0.5 °C.min , ExitT 30 °C.

A possible explanation for the parabolic relationship between yield stress and mixing rate may also be found in the crystallinity. It can be argued that nuclei growth during cooling is dependent upon the agitation of the mass. When mixing is optimal nucleus growth exhibits a similar rate compared to the transport of molecules to and from the nuclei. At lower rate, the supply of molecules that fit in the crystal structure is sub-optimal, while at higher rate the shear leads to abrasive effects. Consequently, an optimum may be observed at 55 rpm. A similar phenomenon has been described for the influence of mixing rate on emulsion polymerization in a batch reactor (15).

When the approach of using a definitive screening design (DSD) is compared to the traditionally used fractional factorial designs, remarkable differences can be observed. For example, in traditional fractional factorial designs, variables are usually studied on two levels while in a DSD variables are studied on three levels. Studying variables on only two levels allows no estimation to be made of curvature, meaning nonlinear patterns of variables. When our results are reflected, it can be seen that in this study every single variable showed nonlinear or curvature effects (**Figure 3**). These patterns would not have been observed in standard two-level screening designs. Thus, a DSD can be viewed as a highly efficient screening design with significant advantages over the more traditionally used fractional factorial designs. Statistical analysis however should be conducted according to the methodology for DSDs (8,16).

Conclusion

This study shows that a definitive screening design is an helpful tool to gain insight into a production process such as the production of cetomacrogol ointment. For this ointment it has been shown that processing had a substantial influence on important rheological parameters such as yield stress. Of all tested process variables, filling temperature and mixing speed are critical. These two variables also have a significant interaction. With these findings a process window was established within which cetomacrogol ointment of constant quality can be produced.

Acknowledgements

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Chapter 3.2

Scale up of ointment manufacturing based on process understanding: from lab to industrial scale

Submitted for publication

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Abstract

The scale up of production processes is a major challenge in pharmaceutical industry. Using a Quality by Design approach upscaling can be based on the design space, which can be assessed on a small scale. In a previous study the critical process parameters were identified by a definitive screening design on cetomacrogol ointment. In the current study, this lab scale (0.5 kg) study was scaled up to industrial scale (2000 kg, filling 100 gram tubes at 75 tubes/min). A similar trend for the influence of filling temperature on ointment consistency (yield stress) was found for lab and industrial scale production. Furthermore, a process window for ointment filling viscosities was established. It was shown that between 26 and 170 Pa.s ointment could be filled into tubes with a low weight variation (<0.5% RSD) resulting in a product with a consistency that meets the pre-set criteria. This approach was subsequently verified using several creams and ointments and showed general applicability.

Introduction

Upscaling is a major challenge in pharmaceutical industry. In order to successfully scale up a process the similarity principle can be adopted. This principle assumes that across all equipment and process scales equal ratios between for example dimensions, forces and temperature gradients are achieved (1). Here, often dimensionless numbers are used as an expression of these ratios. In practice, it is impossible to fully meet the requirement of similarity and therefore scale up is a serious point of attention in drug development (2). One way to approach the upscaling of a process is to do this as much as possible on the basis of process understanding, by using a Quality by Design (QbD) approach (3). The idea is that one should know which parameters can be ignored and which cannot. The knowledge of the critical parameters that really contribute to the final product specifications (critical quality attributes (CQAs)) enables the selection of the appropriate settings at larger scale. Normally, the initial assessment of critical process parameters is conducted on lab scale level since experiments at industrial scale batches are associated with high costs (2).

In ointment production several process parameters may influence the CQAs. A major product property is the consistency, which influences e.g. the spreadability onto the skin. This consistency can be characterized by measuring the yield stress (4,5). In a lab scale (0.5 kg) study it was shown that the yield stress of cetomacrogol ointment was significantly influenced by mixing speed and filling temperature (6).

The effect of mixing and filling temperature on ointment yield stress was studied using a definitive screening design (DSD). This DSD is a statistical method to study the influence of different variables on predefined CQAs. The DSD distinguishes itself from more conventional two-level factorial designs since it allows the study of interactions between variables and detection of curvature in the influence of variables (7). Curvature can only be studied when variables are studied on more than two levels since only then non-linearity in the influence of a variable can be detected. This would also be possible with more conventional designs but, using a DSD, this can be achieved highly efficient using only 2n+1 experimental runs. This results in fewer experiments compared to conventional two level (2^n) or three level (3^n) designs.

The aim of this study was to translate the outcomes of the lab scale design to industrial scale and to establish a process window for an industrial scale filling process.

Material and methods

Materials

The following products were studied: cetomacrogol ointment, cetomacrogol cream and lanette cream II. The composition of these products is shown in **Table 1**. The ingredients were as follows: white petrolatum (Snowwhite N®, Sonneborn international, Amsterdam, the Netherlands), cetomacrogolwax (Galenol® 1618 AE, Sasol GmbH, Brunsbüttel, Germany), isopropylmyristate (Kollicream® IPM, BASF Personal Care and Nutrition GmbH, Düsseldorf, Germany), paraffin oil (110-230 mPa.s, Gustav Heess GmbH, Stuttgart, Germany), colloidal silicon dioxide (Aerosil® 200 vv Pharma, Evonik, Paris, France), sorbic acid (Merck, Darmstadt, Germany), cetiol V (BASF Personal Care and Nutrition GmbH, Düsseldorf, Germany), sorbitol (Neosorb 70/70, Roquette, Vecquemont, France) and lanettewax SX (BASF Personal Care and Nutrition GmbH, Düsseldorf, Germany) Distilled water was prepared by a Elga Centra R 60/120 system (Woodridge, Illinois, USA).

Table 1. Composition of cetomacrogol ointment, cetomacrogol cream and lanettecream II.

Cetomacrogol ointment	Cetomacrogol cream	Lanettecream II
38.2% White petrolatum	15% Cetomacrogolwax	24% Lanettewax SX
25.5% Cetomacrogolwax	0.2% Sorbic acid	0.15% Sorbic acid
15% Isopropylmyristate	20% Cetiol V	16% Cetiol V
21.2% Paraffin oil	4% Sorbitol	4% Sorbitol
0.1% SiO2 particles	60.8% Water	55.85% Water

Rheology

A stress-controlled rheometer (TA instruments HR-2, Etten-Leur, The Netherlands) equipped with a peltier plate and a 40 mm sandblasted parallel plate (TA-instruments plate geometry 40 mm) was used. Approximately 5 gram of ointment was placed on the peltier plate before slowly lowering the upper plate to the preset trimming gap of 1050 µm. After trimming excessive petrolatum the geometry gap was set to 1000 µm.

Temperature ramps were conducted at a heating rate of 1.0 ^{o}C/min between 20 and 70 ^{o}C. Geometry velocity was set at 0.1 rad/s. Yield stress was determined using oscillatory stress sweep (OSS) experiments in a wide stress range (1 – 2000 Pa) at 20°C. Yield stress was defined as the point where the storage and loss modulus lines cross. Data was analyzed using Trios v3.3.0.4055 software.

Ointment filling

Industrial scale filling tests were conducted using a Comadis C1110 with a temperature controlled filling hopper. This is an automated filling machine for pharmaceutical products such as ointments. The filling hopper was filled with ointment (approximately 30 kg per experiment) and conditioned at the required (the temperature required for the experiments) temperature while stirring. Filling rate was set at 75 tubes/min; polyethylene tubes were filled with 100 g of ointment. For every experiment 100 tubes were weighed using a checkweigher (OCS HC-A-2000-2, Kaiserslautern, Germany) for weight variation (%RSD).

Results and discussion

Upscaling cetomacrogol ointment process

Lab scale production of cetomacrogol ointment was studied. The yield stress was used as a measure for the consistency of the product. Batches of 0.5 kg were produced under controlled conditions. The following variables were studied: heating temperature, the addition of SiO_2 particles, mixing rate, cooling rate, filling temperature and isothermal mixing before filling. **Fig. 1** shows dependency of the resulting yield stress for fourteen differently produced cetomacrogol ointment batches.

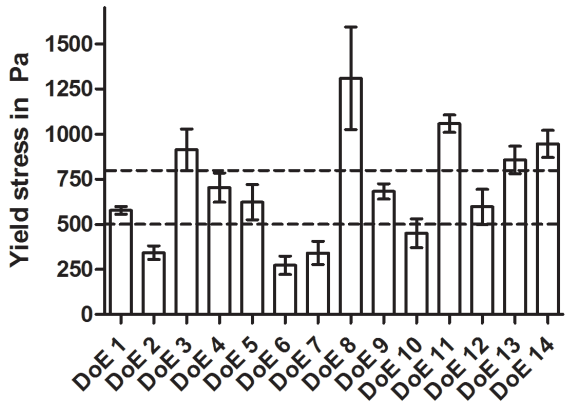

Figure 1. Yield stress results for batches of cetomacrogol ointment measured at 20 °C. CQA window is defined in the range of 500–800 Pa (shown in dashed lines). Yield stress is expressed as mean ±SD. Figure is acquired under the Creative Commons Attribution License (CC BY) from (6).

The yield stress of different batches of cetomacrogol ointment (at 20 °C) was found to lie between 272 and 1309 Pa (**Fig. 1**). To determine the impact of differences in yield stress an internal trained test panel (n=10) was consulted. The experimental values can be roughly categorized as follows; a yield stress <500 Pa corresponds to a cream-like product, values >800 Pa are too thick to be removed from a tube. Dashed lines in **Fig. 1** show that only 5 of the 14 lab scale batches are within these specifications. Clearly, the majority of the batches produced are not within the requirements for the rheological properties. Therefore, it can be concluded that processing in general can substantially influence ointment product characteristics. To assess which variable has a dominating impact on the yield stress, a non-linear statistical model was designed using software from SAS, JMP 12. This is described in more detail by van Heugten et al. (6). The impact of several variables on ointment yield stress is shown in **Fig. 2**.

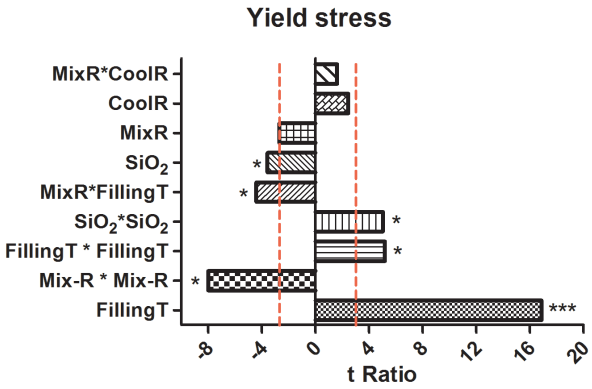

Figure 2. Effect of the formulation and process variables on cetomacrogol ointment yield stress, the sorted parameter estimates. * p-value<0,05, ** p-value<0,001, *** p-value<0,0001. MixR = mixing speed, CoolR = cooling rate, SiO_2 = addition of Aerosil 200 v/v, FillingT = temperature at which containers were filled. The t ratio provides an indication for the significance of an effect. Figure is acquired under the Creative Commons Attribution License (CC BY) from (6). The original "ExitT" was changed in this Figure to "FillingT" the match the accompanying text.

In **Fig. 2** the influence of several variables on cetomacrogol ointment yield stress is shown. On the y-axis in some cases "variable" * "variable" is shown. This, in the case of "variable A * variable B", indicates that two variables have a combined effect on ointment yield stress, or in other words show an interaction. In the case of "Mix-R * Mix-R", this variable shows a non-linear effect, or curvature. This curvature can be observed in the parabolic pattern for the influence of mixing rate on yield stress (shown in (6)). A range of 10-100 rpm was studied and 55 rpm showed the highest yield stress. Clearly, the most significant variables are filling temperature, mixing rate and the addition of SiO_2 particles (p-values: 0.0065, 0.0013 and 0.0073 respectively). Furthermore, mixing rate and filling temperature showed a statistically significant interaction (p-value 0.0116). A more elaborated discussion of the results is described in more detail by van Heugten et al. (6). For the scope of this study, knowing that the mixing rate and filling temperature obviously are the most critical process parameters in the production of cetomacrogol ointment helps in focusing attention during scale up.

Interestingly, on industrial scale production the influence of mixing rate was not found to effect product quality. Especially for a mixing process, the hydrodynamic similarity is important. In the current study a scale up investigation was conducted from a lab scale 1.5 L mixer (ProCept 4M8-Trix) to an industrial scale 2400 L mixer-homogenizer with an additional top-down flow through the homogenizer and pipe (Dinex H2400). The lab

scale mixer is a low shear mixer without a top-down flow, the industrial scale mixer is a low shear mixer with a top-down flow and homogenizer. Clearly, these represent two completely different mixing principles with different Reynolds numbers. The Reynolds number describes flow patterns and is dependent on the vessel diameter and material velocity (1). Most likely, the homogenizer and additional top-down flow on the industrial scale production will greatly influence material velocity. In addition, the homogenizer is likely to play a significant role on the formation of ointment structure on colloidal dimensions due to the high shear forces. Furthermore, the Froude number (Eq. 1) can be used to determine whether the ratio of inertial to gravitational forces is constant for both scales of manufacturing (8), in which n represents the agitator speed measured in revolutions per second, D_a the impeller diameter in meters and g the acceleration due to gravity (9.81 m/s^2).

Equation 1: $$Fr = \frac{n^2 * D_a}{g}$$

For the small scale vessel the Froude number is 0.026 and for the large scale 0.020. These Froude numbers are slightly different which may be another reason why the effect of mixing rate is different on a small compared to a large scale.

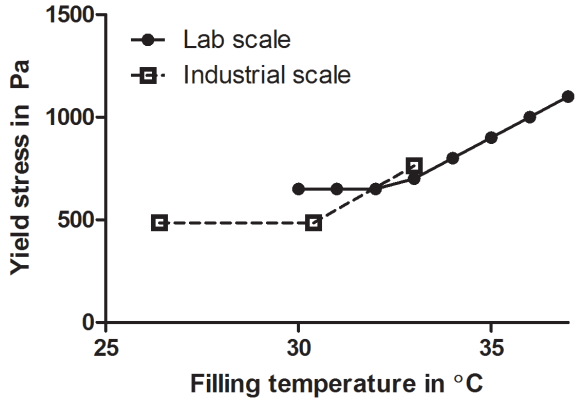

Figure 3. Yield stress at 20 °C for cetomacrogol ointment after filling single tubes at different temperatures on lab scale (0.5 kg). On industrial scale PE tubes of 100 g were filled at a rate of 75 tubes/min.

Fig. 3 confirms that the consistency of the finished product is dependent on the filling temperature during processing. This phenomenon is independent of the manufacturing scale and/or specific equipment used. In **Fig. 3** the data points for temperatures higher than 33 ^{o}C are lacking for the industrial process. Here, the ointment viscosity was found to be too low for operating the filling machine. Therefore higher temperatures were not studied. Clearly, not only the critical influence of filling temperature on ointment yield stress is important when translating lab scale outcomes to industrial scale but, in this case, also processability parameters such as the ointment viscosity.

Process window for industrial scale ointment and cream filling

In order to establish a process window for the ointment filling temperature, additional experiments were conducted. For ointments and creams it is known that their rheological behavior is complex and highly temperature dependent (5,9). The influence of the filling temperature on the weight variation in filled tubes was determined first (**Fig. 4**).

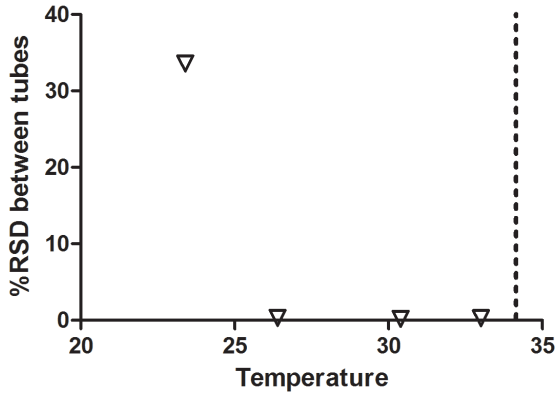

Figure 4. Relationship between cetomacrogol ointment filling temperature and weight variation between filled tubes at industrial scale (expressed as % RSD). The temperature at which ointment was too thin to be filled is shown in a dashed line. All other filling parameters were kept constant during the experiments.

Fig. 4 shows that the variation in tube weight (expressed as % RSD) is highly dependent on ointment temperature. At temperatures somewhere between 24 and 26.4 ^{o}C a high increase in weight variation was found. Between 26.4 ^{o}C and 33 ^{o}C the filling was accurate and reproducible, <0.35% RSD. At 34 ^{o}C however, it was found that ointment was too thin to be filled into tubes. In this situation, ointment splashed from the hopper onto the filling equipment making it impossible to operate the filling machine. The limits for the filling of the cetomacrogol ointment studied here are therefore 26.4 ^{o}C to 33 ^{o}C.

In order to translate these temperatures to viscosities, the relationship between viscosity and temperature for cetomacrogol ointment was studied (Fig 5). Viscosity was studied here in contrast to the aforementioned yield stress. This is due to the fact that the viscosity, in contrast to yield stress, provides information on the materials flow properties that are relevant during mixing and filling. Yield stress on the other hand provides insight into the spreadability of a materials and therefore provides insight into the solid like properties (4).

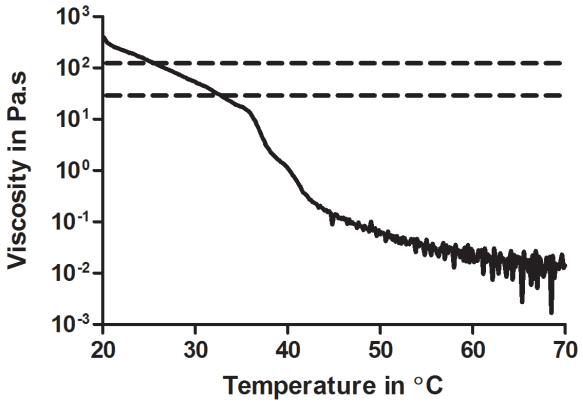

Figure 5. Relationship between temperature and viscosity for cetomacrogol ointment. The process window for filling viscosity is shown in dashed lines.

Fig. 5 shows that with increasing temperature for approximately 35 °C a steep decrease in ointment viscosity can be observed. Temperature clearly has a significant impact on ointment viscosity. Dashed lines correspond to the limits for filling are shown (see also **Fig. 4**). The process window was set from 26.4 – 33.0 °C, the corresponding viscosities are 107 and 26 Pas.

Verification of process window for product viscosity on industrial scale

This process window for product filling was subsequently verified using both creams and ointments, listed in **Table 2**. The same rheometry experiments as are shown in **Fig. 5** were conducted and results are shown in **Table 2**.

Table 2. The process windows that were established using rheometry for a number of products on commercial scale.

Product name	Process window for filling (in °C)
Cetomacrogol ointment	26.4 – 33.0
Cetomacrogol cream	20.0 – 41.3
Lanettecream II	48.0 – 60.0

For all products listed in **Table 2** listed products the temperatures were tested on industrial scale. The verification was determined by testing the upper limit of the filling temperature. For the three products in **Table 2** the shown upper limit was indeed a correct temperature to accurately fill product. No additional data is shown here since verification runs were only conducted for the upper limits. Subsequently the operation specifications were set 2 ^{o}C lower in order to establish a safe operation window. Similar process windows were established and verified for a number of other commercial products that cannot be disclosed due to confidentiality. The process window can thus be translated to different products such as other creams and ointments, assuming the same equipment constraints.

Conclusion

Our study shows that scale up of a production process can be performed based on process knowledge. Thorough characterization of a lab scale process yields information about the most critical of process parameters. Especially these parameters should subsequently be evaluated on industrial scale. In this study it is shown that the mixing rate of the equipment investigated in this study, which is critical on lab scale. Furthermore, the influence of ointment viscosity at filling temperature was shown to be critical for processability on industrial scale with the equipment used in this study. A process window for product viscosity to successfully fill tubes on industrial scale was established for the equipment used in this study. This was shown to be generally applicable to a number of creams and ointments.

Acknowledgements

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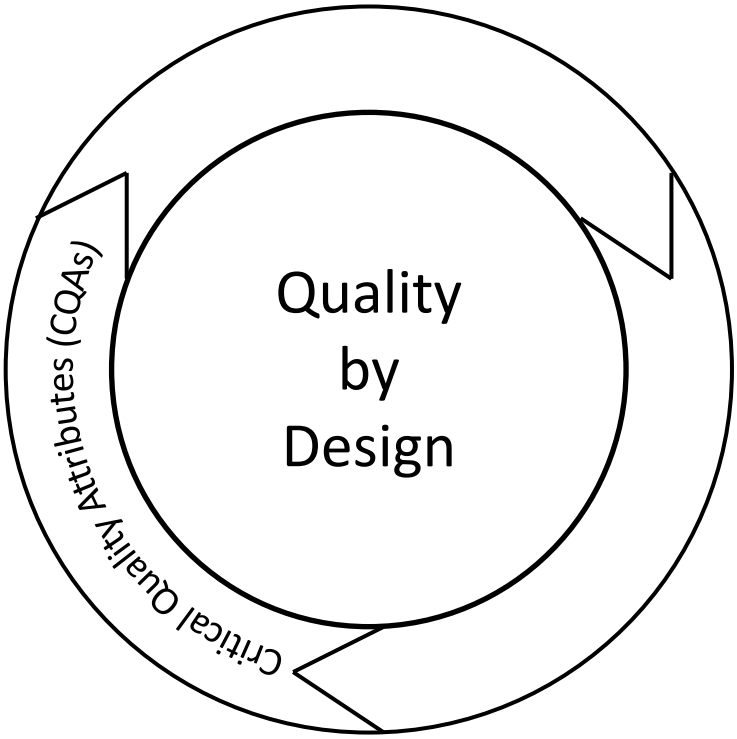
Chapter 4

Critical Quality Attributes (CQAs)



Chapter 4.1

Development and validation of a stability-indicating HPLC-UV method for the determination of triamcinolone acetonide and its degradation products in an ointment formulation

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Abstract

A stability indicating high performance liquid chromatography method has been developed for the determination of triamcinolone acetonide (TCA) and its main degradation products in ointment formulations. The method, based on extensive stress testing using metal salts, azobisisobutyronitrile, acid, base and peroxide, showed that TCA undergoes oxidative degradation. All degradation products were identified using HPLC mass spectrometry. Separation and quantification was achieved using an Altima C18 RP18 HP column (250 x 4.6 mm^2, with 5 µm particles) using a mobile phase consisting of acetonitrile and water buffered at pH 7 using 10 mM phosphate buffer. A gradient mode was operated at a flow rate of 1.5 ml/min and detection was at 241 nm. The method showed linearity for TCA and impurity C in 0.02–125% of the workload, both square roots of the correlation coefficients were larger than 0.9999. Repeatability and intermediate precision were performed by six consecutive injections of both 1.25% and 125% of the work load for both TCA and impurity C divided equally over two days. RSD were 0.6% and 0.7% for TCA and 0.5% and 0.1% for impurity C respectively. Accuracy was determined as well, the average recoveries were 99.5% (±0.1%, n=3) for TCA and 96.9% (±1.3%, n=3) for impurity C respectively from spiked ointment samples. The robustness was also evaluated by variations of column (old vs new), mobile phase pH and filter retention. The applicability of the method was evaluated by analysis of a commercial ointment formulation. Interestingly, the extensive stress tests were able to predict all degradation products of TCA in a long term stability ointment sample.

Introduction

Triamcinolone acetonide (TCA) is a synthetic glucocorticosteroid with immunosuppressive and anti-inflammatory activity. It binds in the target cell to specific cytosolic glucocorticoid receptors and subsequently interacts with glucocorticoid receptor response elements on DNA thereby altering gene expression (1). TCA has been used for over fifty years and is still frequently prescribed in the treatment of several skin diseases like eczema and psoriasis. It is used in many cream and ointment formulations, including an ointment that is widely used in the Netherlands: TCA ointment FNA (Formulary of Dutch Pharmacists).

Because of the widespread use of TCA in varying matrices several chromatographic methods for the analysis of the compound have been described (2–9). These methods are suitable for the determination of the TCA content, but not for the quantification of degradation products. The quantification of degradation products is essential in stability research of pharmaceutical products following the ICH Q2 (R1) guideline (10). Additionally, the degradation of TCA is poorly described in literature. As a consequence, currently no stability indicating method (SIM) for TCA ointment FNA is available.

To develop and validate a method specificity, stressed samples are essential. According to the ICH Q2 (R1) guideline stressed samples should be created using heat, humidity, acid, base, oxidation and light stress (10). The vagueness of this guideline leads to a variety of experience-based approaches which are often not comprehensive in their predictability. The scientific background and practical implementation of adequate stress testing is described extensively elsewhere (11). Since oxidation is the predominant mechanism of TCA degradation a comprehensive set of oxidative stress testing should be used to attain a more comprehensive prediction of the profile of degradation products (2). Therefore, we incorporated not only a peroxide (e.g. hydrogen peroxide [H_2O_2]), but a radical initiator (e.g. azobisisobutyronitrile [AIBN]) and trace metals (e.g. iron and copper salts) into the set of stress tests (12,13). All other conditions as mentioned in the ICH-guideline were implemented as well. To show specificity, a four and a half year old ointment sample was used. Degradation product identification was performed in order to assist in method development.

The aim of this study was to develop and validate a HPLC-UV SIM for TCA ointment FNA. In support of this aim TCA degradation products were identified using LC-MS after evaluating the outcome of the set of stress tests.

Material and methods

Reagents and chemicals

HPLC grade acetonitrile (ACN), dichloromethane, methanol (MeOH) and hexane were obtained from Avantor Performance Materials (Center Vally, Pennsylvania, USA). Distilled, deionized water was prepared by an Elga Centra R 60/120 system (Woodridge, Illinois, USA). Copper(II) acetate was obtained from Alfa Aesar (Havehill, Massachusetts, USA). Disodium edetate, hydrogen peroxide (H_2O_2), iron(III) chloride ($FeCl_3$), copper(II) chloride ($CuCl_2$), sodium phosphate monobasic (NaH_2PO_4), sodium phosphate dibasic (Na_2HPO_4) and azobisisobutyronitrile (AIBN) were obtained from Merck (Darmstadt, Germany). Propylene glycol (PG) was obtained from Brenntag (Dordrecht, The Netherlands). 1 M hydrogen chloride (HCl) and 0.01 M sodium hydroxide (NaOH) solutions were prepared on site.
TCA ointment FNA consists of 0.1% TCA, 10% PG, 10% lanolin and 79.9% petrolatum.

LC-MS analysis

MS was conducted on a Micromass Quattro Ultima TQD system equipped with an electrospray ionization (ESI) source (Waters Chromatography, Etten-Leur, The Netherlands). Masses were scanned from *m/z* 50-1100, gas flow to 530 L/hr, gas temperature to 350 °C and voltage 3 kV. Data was analyzed with Masslynx version 4.0 software. The mobile phase components were ACN and water buffered at pH 6.8 using 12 mM ammonium acetate.

To attain insight in the product specific degradation products, a four and a half year old 0.1% TCA ointment FNA sample that was stored throughout its shelf life at room temperature in aluminum tubes was used. The sample was extracted using the sample preparation method provided in section 2.6 and analyzed using the settings described above. Mass spectra are included in supplementary data.

HPLC-UV

Chromatography was conducted on a Shimadzu Prominence-i LC-2030C 3D liquid chromatograph with diode array detector (Kyoto, Japan) and an Altima C18 RP18 HP column (250 x 4.6 mm^2, with 5 µm particles) (Mandel Scientific Company, Ontario, Canada). The flow rate was 1.5 ml/min and UV detection was at 241 nm. Mobile phase components were ACN and water buffered at pH 7 using 10 mM phosphate buffer. Injection volume was 20 µl. Chromatograms were obtained and analyzed with Shimadzu

LabSolutions software version 5.5.7. A gradient program was run: 0% ACN from start to 12 minutes, increased to 32% ACN at 12 minutes, maintained at 32% until 30 minutes, increased to 70% at 40 minutes, decreased to 0% at 42 minutes and maintained at 0% until 47 minutes.

Synthesis of impurity C

The synthesis of impurity C (compound 2, **Figure 1**) was based upon a method described in literature (14). Impurity C was synthesized by dissolving 600 mg TCA and 31.5 mg copper(II)acetate in 150 ml MeOH. Air was bubbled through the solution for 60 minutes. The reaction was quenched by adding 20 ml of 2.5 mg/ml disodium edetate aqueous solution. The solution then was concentrated to 30 ml under cold air and was extracted twice with 200 ml dichloromethane. Finally, the dichloromethane was evaporated under cold air to yield impurity C.

Stress testing

Stress testing was performed on 0.5% solutions of TCA in PG. These solutions were exposed to the conditions described in **Table 1**. Conditions were chosen based on a degradation target of 5-20%. HCl and NaOH were used to simulate acid and base catalyzed degradation. AIBN, H_2O_2 and $FeCl_3$ and $CuCl_2$ were used to simulate radical initiator, peroxide and trace metal mediated oxidation respectively. Light stress was omitted because of irrelevancy as TCA is protected against light in the product by its container.

Sample preparation

Ointment samples were dispersed in hexane and extracted with ACN and water buffered at pH 7 using 10 mM phosphate buffer (1:1). PG solutions were diluted with ACN or ACN-buffer (1:1). Synthesized impurity C was dissolved in ACN or ACN-buffer (1:1). TCA references were dissolved in ACN-buffer (1:1).

Method validation

The method was validated according to the ICH Q2 (R1) guideline. Accuracy, precision (including both repeatability and intermediate precision), specificity, linearity, range and detection and quantification limits (LOD and LOQ) were assessed. Appropriate stressed samples were used for the assessment of specificity and resolution. Stressed samples were appropriate if they showed a degradation between 5 and 20%. Compounds were taken into account if they were present in a concentration of ≥1.0%.

Accuracy

For accuracy a freshly prepared ointment matrix was spiked with TCA and impurity C in concentrations of 100% and 1% of the work load respectively. Recovery was determined on three consecutive runs.

Precision

For precision repeatability and intermediate precision were performed by using six consecutive injections of both 1.25% and 125% of the work load for both TCA and impurity C in ACN-buffer (1:1) divided equally over two days.

Specificity

The four and a half year old ointment was used to determine the method specificity by determination of the smallest resolution between any two peaks after sample preparation.

Linearity, range, LOD and LOQ

Linearity for 0.020, 0.1, 0.5, 2.5, 12.5, 62.5 and 125% of the workload was determined for both TCA and impurity C. The LOD and LOQ were determined by linear regression analysis.

Robustness

The robustness was determined using a number of test. Firstly, an old and a new column of the same type were compared for resolution between TCA and impurity C. Secondly, the influence of mobile phase pH on the resolution between TCA and impurity C was tested by using a mobile phase at pH 6.5 and 7. Thirdly, the TCA and impurity C recoveries were determined after filtration by analyzing three injections of 125% of the workload before and after filtration.

Results and discussion

Degradation product identification

An ointment that was stored at room temperature in the drug product container for four and a half years was used to identify the degradation products that may form in the ointment. HPLC-MS analyses were conducted to determine the mass of degradation products. Impurity C (m/z: 451.4), a C_{17}-carboxylic acid (m/z: 421.4), a PG hemi-acetal of impurity C (m/z: 509.2), a C_{17}-glyoxilic acid (m/z: 449.5) and a PG ester of the C_{17}-glyoxilic acid (m/z: 507.5) (**Figure 1**; compounds 2, 3, 4, 5 and 6 respectively) were identified as degradation products based on mass. **Figure 1** presents TCA and the five identified degradation products. Comparable degradation products of impurity C and C_{17}-carboxylic acid are described in literature as degradation products of TCA and hydrocortisone (2,15). The PG hemi-acetal is described in literature as a comparable degradation product of triamcinolone acetophenonide in the presence of PG (16). The C_{17}-glyoxilic acid has been described before for flurandrenolide in a cream formulation only, but not for TCA or other described corticosteroids (2,14,15,17). For the PG ester of the C_{17}-glyoxilic acid no prior report was found. However it seems logical that such an acid may form an ester when (di-)alcohols such as PG are present. Furthermore, the C_{17}-carboxylic acid and the C_{17}-glyoxilic acid showed a shift in retention time in response to mobile phase pH.

Optimization of chromatographic conditions

The starting point of the optimization was the TCA related substances method of the European Pharmacopoeia (monograph 0533). To use this method for the TCA ointment FNA an extraction procedure was added to the sample preparation. Extraction of the ointment was performed by dispersing it in hexane followed by extraction using ACN as extraction solvent.

In the Pharmacopoeia method the C_{17}-carboxylic acid (compound 3) showed poor retention and peak symmetry. Therefore this method was adapted and further developed. The retention was improved by changing the gradient program from 32 to 0% ACN at the start of the program. Peak symmetry was improved by buffering the aqueous mobile phase at pH 7 using 10 mM phosphate buffer.

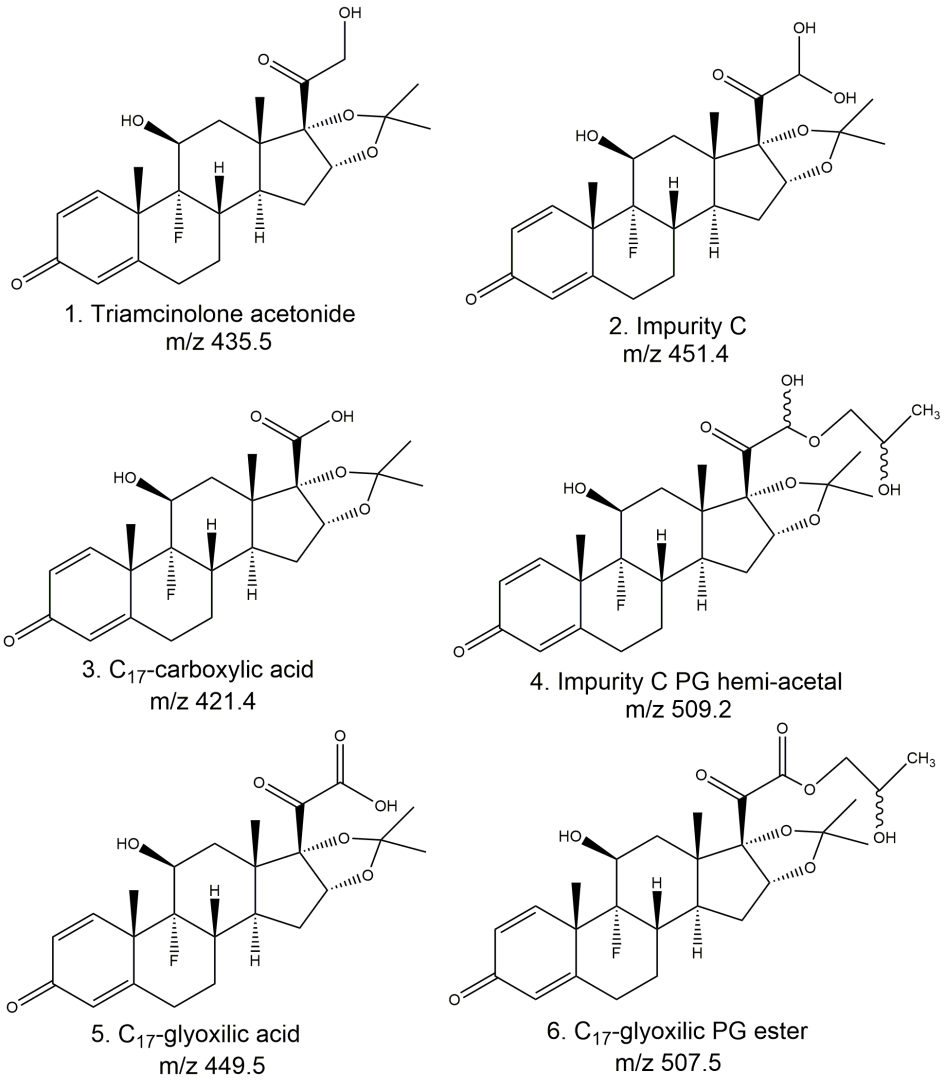

Figure 1. Molecular structures of triamcinolone acetonide (TCA) and its most predominant degradation products. Structures are based on literature (15,16) in combination with mass determination using HPLC-MS. 1: TCA 2: impurity C 3: C_{17}-carboxylic acid 4: impurity C propylene glycol hemi-acetal, 5: C_{17}-glyoxilic acid and 6: C_{17}-glyoxilic acid propyleneglycol ester.

During injection in the HPLC the sample was diluted with water. This led to the conversion of the PG hemi-acetal to impurity C during analysis due to an abundance of water. This is reflected in a broad impurity C peak that impaired proper integration was the result. This issue was resolved by changing the extraction solvent from ACN to ACN-water buffered at pH 7 with 10 mM phosphate buffer (1:1). In this way, the PG hemi-acetal converted to

impurity C prior to injection. **Figure 2** shows the chromatograms of synthesized impurity C, dissolved in PG and diluted fifty times with ACN or ACN-water buffered at pH 7 with 10 mM phosphate buffer.

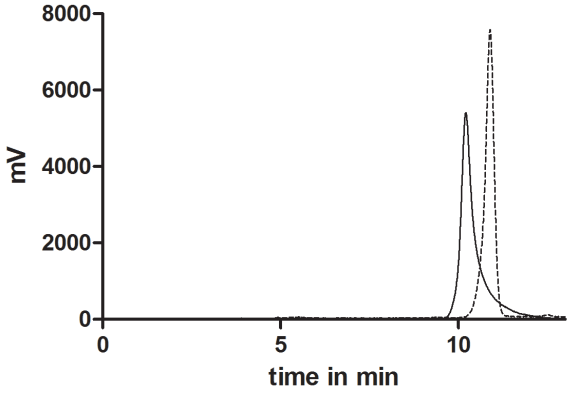

Figure 2. Chromatograms of synthesized impurity C dissolved in propylene glycol (PG) and diluted with either acetonitrile (ACN) (black) or ACN-water buffered at pH 7 with 10 mM phosphate buffer (dashed line).

In **Figure 2** it is clearly shown that changing the extraction solvent to ACN-water buffered at pH 7 improves the peak shape. Obviously, in the presence of water a hemi-acetal such as compound 4 converts to its aldehyde hydrate form (impurity C), this conversion is shown in **Figure 3**. Similar equilibria have been described before (18), and have shown to be dependent on both the alcohol and aldehyde. Therefore, it is likely that when a different solvent is used in a comparable product this equilibrium differs from what we have seen in this study.

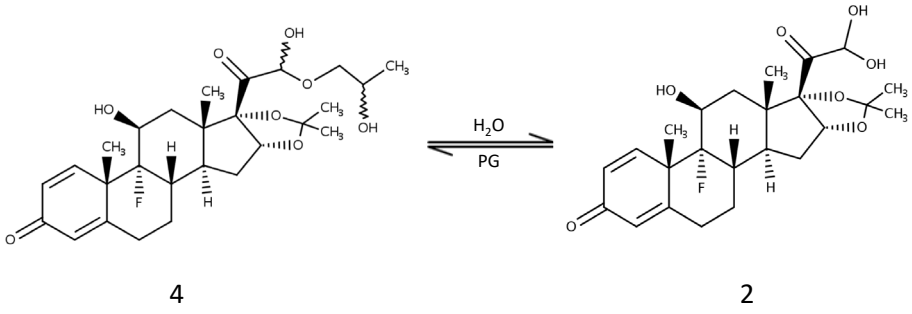

Figure 3. Equilibrium between impurity C propylene glycol (PG) hemi-acetal (compound 4) and impurity C (compound 2).

Stress testing results

Stress tests were conducted in PG as a model for the ointment. Since the solubility of TCA in PG is >100 times higher than its solubility in lanolin and petrolatum it can be assumed that TCA is predominantly present in the PG phase and that degradation of TCA will consequently take place in the PG phase within the ointment. **Table 1** presents the results.

Table 1. Stress testing results. All solutions contained 0.5% triamcinolone acetonide (TCA) in propylene glycol (PG). AIBN: azobisisobutyronitrile. 1: TCA; 2: impurity C; 3: C_{17}-carboxylic acid, 4: impurity C propylene glycol hemi-acetal, 5: C_{17}-glyoxilic acid and 6: C_{17}-glyoxilic acid propylene glycol ester.

Stress condition				Amount of compound after stress (% of total)				
#	Medium	Temp.	Time	1	2/4	3	5	6
1	TCA ointment	25 °C	4.5 y	76	4.8	9.2	1.2	8.5
2	5 mM HCl	60 °C	7 d	99	-	1.1	-	-
3	0.5 mM NaOH	60 °C	4 d	85	8.1	6.0	-	-
4	11.5 mM AIBN	60 °C	7 d	96	-	-	-	1.8
5	3% H_2O_2	25 °C	7 d	99	-	-	-	-
6	5 mM $FeCl_3$	60 °C	7 d	91	5.5	1.8	1.2	-
7	5 mM $CuCl_2$	60 °C	7 d	98	-	-	-	-

Table 1 presents an overview of the outcomes of the stress studies. Firstly, six degradation products were found in the aged TCA ointment. However, compound 4 is detected as compound 2 as described in section 3.2 and thus this degradation product is described as compound 2/4 in **Table 1**. Secondly, it can be seen that in acidic conditions, in AIBN, peroxide and $CuCl_2$ only minor degradation occurred but in alkaline conditions or when $FeCl_3$ was present degradation was more extensive. Corticosteroids in alkaline conditions have been described before to study degradation patterns (15,17,19). $FeCl_3$ however is more uncommon to use in corticosteroid degradation studies. Thirdly, by combining conventional stress tests (acid, base and peroxide) with the more unconventional AIBN, $CuCl_2$ and $FeCl_3$ (as recommended in literature (12,13)) all degradation products that formed in the actual drug product were found.

Method validation results

A chromatogram of the four and a half year TCA ointment sample is shown in **Figure 4**. In this chromatogram the identity of the peaks is marked with numbers corresponding to the degradation products in **Figure 1**.

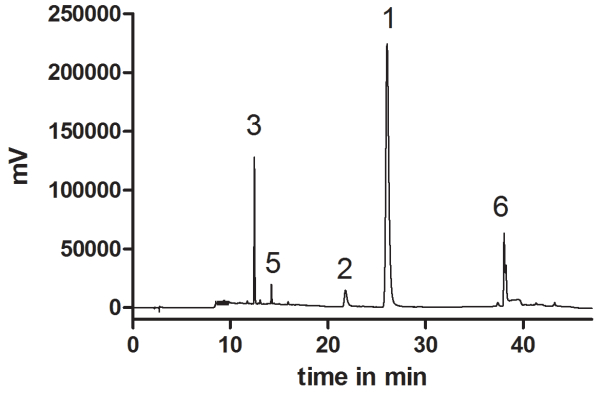

Figure 4. Chromatogram of a four and a half year sample of 0.1% TCA ointment FNA stored at room temperature throughout shelf life. The peaks are marked with numbers corresponding to the components presented in Figure 1.

The method validation results are summarized in **Table 2** and further discussed in the following paragraphs.

Table 2. Summary of method validation results, TCA = triamcinolone acetonide Imp. C = impurity C, if applicable (SD) is presented for n=3.

	Description of test	Results
Accuracy	Recovery	TCA: 99.5% (0.1); Imp. C: 96.9% (1.3)
Precision	%RSD for 1.25% and 125% of workload	TCA: 0.6% and 0.7%; Imp. C: 0.5% and 0.1%
Specificity	Resolution	>7.1
Quantification limits	LOD	TCA: 0.008%; Imp. C: 0.011%
	LOQ	TCA: 0.025%; Imp. C: 0.032%
Robustness	Resolution old vs new column	6.5 (0.0) (old) vs 7.1 (0.0) (new)
	Resolution mobile phase pH 6.5 vs 7.5	4.5 (0.15) (low pH) vs 6.5 (0.0) (high pH)
	Tailing factor C_{17}-carboxylic acid for pH 6.5 vs pH 7.5	1.45 (0.1) (low pH) vs 5 (0.0) (high pH)
	Recovery after filter retention	TCA: 100% (0.4); Imp. C: 98.9% (0.2)

Accuracy

Recovery of TCA and impurity C from a freshly prepared ointment matrix was determined three consecutive times. Mean (standard deviation [sd]) recovery from the ointment was 99.5% (0.1%, n=3) for TCA and 96.9% (1.3%, n=3) for impurity C respectively.

Precision

RSD for 1.25% and 125% of the workload were 0.6% and 0.7% for TCA and 0.5% and 0.1% for impurity C respectively.

Specificity

Stressed sample 1 (**Table 1**) was used during the specificity assessment, because all degradation products were present. Resolution between impurity C and TCA was 7.1. Resolutions amongst other components were greater than 7.1. Matrix components showed no interfering peaks.

Linearity, range, LOD and LOQ

The equation of the calibration curves were y = 76523x + 11062 with R^2 = 0.99998 for TCA and y = 70276x + 13552 with R^2 = 0.99995 for impurity C. **Figure 5** presents the calibration curves. LOD and LOQ were 0.008% and 0.025% for TCA and 0.011% and 0.032% for impurity C respectively.

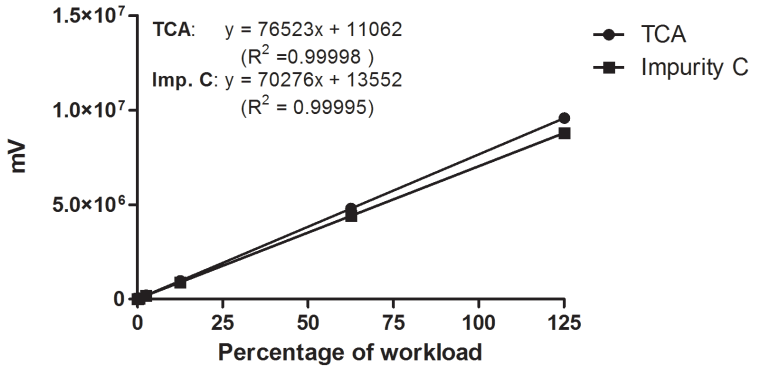

Figure 5. Triamcinolone acetonide (TCA) and impurity C (Imp. C) calibration curves for 0.02 – 125 % of the work load.

Robustness

Robustness with respect to the column was performed by three consecutive injections of stressed sample 1 (**Table 1**) on both an old and a new column of the same type. Mean (sd) resolutions between TCA and impurity C using the old and new column were 6.5 (0.0, n=3) and 7.1 (0.0, n=3) respectively.

Robustness with respect to the mobile phase pH was performed by three consecutive injections of stressed sample 1 using mobile phase buffered at pH 6.5 and 7.5. Mean (sd) resolutions between TCA and impurity C for pH 6.5 and 7.5 were 4.5 (0.15, n=3) and 6.5 (0.0, n=3) respectively. Mean (sd) tailing factors of C_{17}-carboxylic acid for pH 6.5 and 7.5 were 1.45 (0.1, n=3) and 5(0.0, n=3) respectively.

Robustness with respect to filter retention was performed by three injections of 125% of the work load using both filtered and unfiltered samples. Mean (sd) recoveries for the filtered and unfiltered were 100% (0.4%, n=3) for TCA and 98.9% (0.2%, n=3) for impurity C respectively.

Conclusion

A stability indicating HPLC-UV method was developed, validated and applied to a relevant pharmaceutical ointment, TCA ointment FNA. During the development degradation products were identified and an innovative method to influence a hemi-acetal aldehyde hydrate balance was found in adapting the extraction solvent. Only by using uncommon stress tests (AIBN, $CuCl_2$ and $FeCl_3$) all degradation products that were found in the actual product after long term storage were found. Thereby indicating the importance of comprehensive oxidative stress test designs in pharmaceutical development. The described method can be used in practice for TCA ointment FNA and potentially for other ointments as well.

Acknowledgements

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Chapter 4.2

The role of excipients in the stability of triamcinolone acetonide in ointments

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Abstract

Degradation of triamcinolone acetonide (TCA) in an ointment was investigated. TCA appeared to be concentrated in propylene glycol (PG) which in turn is dispersed in a lanolin-petrolatum mixture. Two predominant degradation products were identified: a 21-aldehyde and a 17-carboxylic acid. The 21-aldehyde is formed after TCA is oxidized by O_2, a reaction that is catalyzed by trace metals. Logically, the content of trace metals has a profound effect on the degradation rate. It was shown that trace metals are extracted from lanolin and petrolatum by PG, increasing the concentration in PG. In accordance with these findings, TCA degrades faster in PG that is present in the ointment formulation than in regular PG. The 21-aldehyde was confirmed to be a primary degradation product, while the 17-carboxylic acid was identified as a secondary degradation product. Based on the mechanism of degradation the ointment can be stabilized by the addition of sodium metabisulfite which was shown to reside also in the PG phase within the ointment.

Introduction

Corticosteroids are widely used in a broad range of products. A selection of these products are for dermal application, such as ointments. In the Netherlands TCA ointment 0.1 % FNA (Formulary of Dutch Pharmacists) was available until it was withdrawn from the market after license-holders reported poor chemical stability (1). The ointment consists of 0.1 % TCA, 10 % propylene glycol (PG), 10 % lanolin and 79,9 % petrolatum. Various similar products are used worldwide.

TCA and molecularly similar corticosteroids are prone to oxidative degradation. This particular degradation predominantly occurs at the 17-side chain of the corticosteroid molecules (2–5). Amongst other degradation products, two are the most often mentioned for corticosteroids with the same 17-side chain as TCA (**Figure 1**); first a 21-aldehyde degradation product which formation is described in aqueous and alcoholic solutions (**Fig. 1**, compound 2). This 21-aldehyde is formed by a reaction between TCA and molecular oxygen (O_2) that is catalyzed by metal salts (4). Second, for hydrocortisone and flurandrenolide the formation of a 17-carboxylic acid in alkaline environment was demonstrated by using O_2 and OH^- as reagents (2,3). Other degradation products concern degradation of the A ring (6–8) or hydrolysis of the acetonide moiety (9). For a water-free environment such as the TCA ointment it was shown that the 21-aldehyde and 17-carboxylic acid are the main degradation products (10). The mechanism by which these are formed remains unclear however.

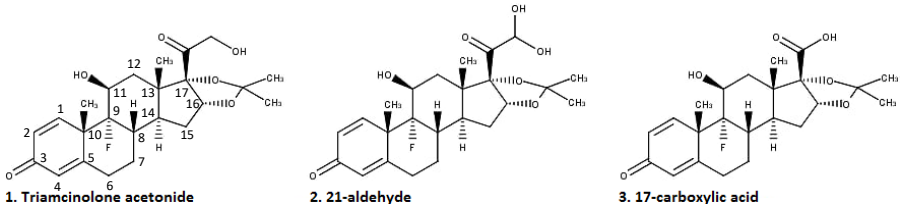

Figure 1. Triamcinolone acetonide (TCA) and two degradation product as described for TCA and similar corticosteroids (2–5).

The aim of this study was to investigate the degradation mechanism of TCA in TCA ointment 0.1% FNA.

Material and methods

Chemicals and reagents

The following chemicals and reagents were used (all materials complied with the quality requirements of the European Pharmacopoeia (Ph. Eur. 2016)): TCA (Newchem, Milan, Italy), PG (Brenntag, Dordrecht, the Netherlands), lanolin (Stella Lanolines, Mouscron, Belgium), petrolatum (Sonneborn, Amsterdam, the Netherlands), copper(II) acetate ($Cu(Ac)_2$) (Alfa Aesar, Haverhill, MA, USA), tert-butyl peroxybenzoate (TBPB), sodium metabisulfite and 1,10-phenantroline (Sigma-Aldrich, St Louis, MO, USA), hexane and acetonitrile (Avantor Performance Materials, Center Valley, PA, USA) and phosphoric and acetic acid (Boom, Meppel, the Netherlands). Distilled, deionized water was prepared by an Elga Centra R 60/120 system (Woodridge, IL, USA).

The solubility of TCA in the ointment components and microscopical structure of TCA ointment

100 g of PG was added to an Erlenmeyer flask and heated to 60 °C on a magnetic stirrer hot plate (IKA C-MAG HS4, Staufen, Germany). TCA was added in 0.25 g increments. 100 g of lanolin or petrolatum was added to stainless steel mortars that were heated to 60 °C on a water bath (Ika Werke HB4 Basic, Strufen, Germany). TCA was added in 0.010 g increments. End point (solubility) was defined as the amount of TCA where solid dispersed particles were still observed after intensive stirring for 10 minutes.

Additionally, TCA ointment sample was analyzed for microscopic structure using a light microscope (Euromex ME-2880 microscope, Euromex, Arnhem, The Netherlands) with a magnification of 40x.

The metal content in the excipients of the ointment

PG was mixed with lanolin and petrolatum in a ratio of 1:1:8 (i.e. the ratio of the ointment formulation). This mixture was stored at 60 °C for one month. At 60 ^{o}C the ointment is phase separated in a PG and a lanolin-petrolatum phase. The PG phase was separated from the lanolin-petrolatum phase by using a syringe. 0.5 g of this PG extract, normal PG, lanolin and petrolatum were dispersed in 10 ml nitric acid and 2 ml of hydrogen peroxide in a Teflon tube in a MDS 2000 lab microwave (CEM Corporation, Matthews, NC, USA). After completing the microwave program (15 min 60 % power and 80 psi; 15 min 80 % power and 100 psi; 30 min 90 % power and 120 psi) the sample tubes were cooled, transferred to 50 ml sample tubes and diluted to 30 g with water. Samples were

analyzed using an inductively coupled plasma optical emission spectrometry (ICP-OES) spectrometer Dual-View Prodigy 7 (Teledyne Leemanlabs, Hudson, NH, USA) for iron, nickel and copper content.

LC settings

LC was conducted on a Shimadzu Prominence-i LC-2030C 3D liquid chromatograph with diode array detector (Kyoto, Japan) and an Altima C18 column (250 x 4.6 mm, with 5 µm particles) (Mandel Scientific Company, Ontario, Canada). The flow rate was 1.5 mL/min, the injection volume was 20 µL and UV detection was performed at 241 nm. The mobile phase consisted of acetonitrile and water with the addition of 20 mM phosphoric acid (acetic acid when subsequently analyzed with MS). A gradient program was used: 0% acetonitrile from start to 12 minutes, increased to 32% at 12 minutes, maintained at 32% until 30 minutes, increased to 65% at 40 minutes, decreased to 0% at 42 minutes and maintained at 0% until 47 minutes. Chromatograms were obtained and analyzed with Shimadzu LabSolutions software version 5.5.7. This method is similar to a recently published analytical method for TCA ointment (10).

MS settings

MS was conducted on an Agilent 1100 series ion-trap system equipped with an electrospray ionization (ESI) source and liquid chromatography sprayer (Agilent Technologies, Waldbronn, Germany) and operated in positive mode. Masses were scanned from m/z 50-600, nebulizer pressure was 70 psi, gas flow was 12 L/min, gas temperature was 350 °C and capillary voltage was 1600 V. Data was analyzed with Agilent LC/MSD Trap 4.1 version 5.0 (build 65) software.

Results and discussion

The microscopical structure of the TCA ointment and the solubility of TCA in ointment excipients

Using a light microscope the physical structure of the ointment appeared to consist of two phases; a dispersed phase in a more voluminous continuous phase. Since PG is more polar than lanolin and petrolatum, it seems logical to state that the dispersed phase consists of PG and the continuous phase of lanolin and petrolatum. The latter two are mixable, while these compounds hardly mix with PG. To study in what phase TCA resides within the ointment, solubility tests in the separate ingredients were conducted. The solubilities of TCA in PG, lanolin and petrolatum were 1.25, <0.01 and <0.01% respectively. From this it can be concluded that TCA is mainly present in the PG phase that is emulsified in a lanolin-petrolatum mixture. Therefore, degradation experiments in PG can be assumed to yield representative outcomes.

The identity of the major TCA degradation products in the ointment

To elucidate the degradation mechanism of TCA, degradation products in the ointment were determined. Ointment was prepared and stored in closed glass containers at 60 ^{o}C for 1 month. Samples were analyzed using LC-MS. The two major degradation products were identified to be the 21-aldehyde and the 17-carboxylic acid (**Fig. 1**, compounds 2 and 3 respectively) based on m/z ratio's of 451.2 and 421.2 respectively. The 17-carboxylic acid showed a shift in retention time in response to mobile phase pH confirming it to be an acidic compound. To confirm that testing at 60 °C shows a realistic degradation pattern, an old ointment that was stored for 5 years at room temperature was tested; the same degradation products were formed.

The degradation mechanism of TCA

The two degradants, 21-aldehyde and 17-carboxylic acid, are oxidative degradation products of TCA. They have been described before as degradants of hydrocortisone and TCA in aqueous and ethanolic solution in the presence of O_2 and metal salts (2–4). For a water free environment it is unclear what reactants are present to oxidize TCA. In the ointment, the excipient lanolin is known to contain peroxide impurities (European Pharmacopoeia monograph 0134). Furthermore, the presence of trace metals cannot be excluded since excipients of natural origin are generally known to contain low levels of these (11). Therefore, a stress study was conducted to investigate whether TCA is oxidized by peroxide residues and if its oxidation is catalyzed by trace metals in the non

aqueous ointment. TBPB was used as a model for organic peroxide residues (12). TCA was dissolved in PG and exposed to varying combinations of $Cu(Ac)_2$, purging air and TBPB (**Table 1**). TCA content was determined after storage at 60°C for 7 days.

Table 1. TCA content after storage at 60 °C for 7 days after exposure to 6 combinations of copper(II) acetate ($Cu(Ac)_2$) (0.16 mM, 10 ppm), purging air and tert-butyl peroxybenzoate (TBPB) (0.15 M, 3%).

$Cu(Ac)_2$	Air	TBPB	TCA content (in % ±RSD)
-	-	-	99.5 (±0.01)
-	+	-	99.1 (±0.01)
-	-	+	99.8 (±0.00)
+	-	-	80.3 (±0.02)
+	+	-	78.3 (±0.00)
+	-	+	96.1 (±0.10)

The results in **Table 1** clearly show that without the presence of $Cu(Ac)_2$ no clear degradation of TCA occurred. This indicates that the presence of air or peroxides alone does not initiate TCA degradation. However, when TCA was exposed to $Cu(Ac)_2$ significant decomposition occurred. This is slightly increased by purging air in the presence of $Cu(AC)_2$. Previously, it has been shown that TCA degradation is oxidative (2–4) and therefore oxygen must be present in PG to react with TCA. To study whether sufficient oxygen is present in PG, it was purged for one hour with nitrogen in the presence of $Cu(Ac)_2$ in preliminary experiments (90.3% TCA left for the purged and 85.9% for the untreated sample). Clearly, less degradation occurred in the purged PG compared to untreated PG. This clearly indicates that oxygen is indeed necessary for TCA to decompose and that in untreated PG sufficient oxygen is present to react with TCA.

Interestingly, the combination of $Cu(Ac)_2$ and TBPB led to less degradation than $Cu(Ac)_2$ alone (96.1% versus 80.3%). A similar amount of decomposition was expected since the same amount of $Cu(Ac)_2$ was present in the sample. It therefore appears that the peroxide (TBPB) and $Cu(Ac)_2$ interact. Such interaction can possibly be explained by the Fenton-type reaction that occurs when trace metals (M) react with peroxides (see Eq. 1 and 2). Competition for $Cu(Ac)_2$ between TBPB and TCA can explain the difference in degradation between the samples with $Cu(Ac)_2$ alone and those with both $Cu(Ac)_2$ and TBPB. Moreover, the hydroxyl radicals (HO•) that form during Fenton-type reactions are much more reactive than the natural oxidant (ROO•) (11,13). Even in this environment TCA degraded less than in the presence of $Cu(Ac)_2$ alone, affirming TCA oxidation is mediated by trace metals and oxygen rather than peroxides.

Equation 1: $M + H_2O_2 \rightarrow M^+ + HO\bullet + OH^-$

Equation 2: $M^+ + H_2O_2 \rightarrow M + HOO\bullet + H^+$

Influence of storage conditions

As is shown above the presence of air enforces TCA degradation. Therefore, an in use stability study was performed on a 0.5% TCA in PG:lanolin (1:1) mixture stored in closed glass containers and in glass containers that were opened twice a week for 10 minutes. After 218 days at 60 °C 96.8 % (±0.2) of TCA (±RSD) was left in the closed containers whilst in the opened containers 73.1% (±0.9) was left (n=3). Clearly, TCA decomposes significantly faster in opened containers. When containers are opened the sample is exposed to fresh air, allowing the supply of oxygen. Since in general oxygen is more soluble in fatty environment compared to polar environment (14), it seems likely that opening containers twice a week is enough to replenish the oxygen concentration in the sample. Therefore, more substrate (oxygen) is available to react with TCA which can explain the higher TCA degradation.

The influence of trace metal content on TCA degradation constant

Metal salts greatly influence TCA degradation (4). To determine the relation between trace metal content and TCA degradation, a 0.5 % TCA solution in PG was exposed to varying amounts of $Cu(Ac)_2$ and equilibrated with air. $Cu(Ac)_2$ was used as a model for other metal salts which are likely to react in a similar way (2,4). TCA content was determined after one month. **Fig. 2** presents the results.

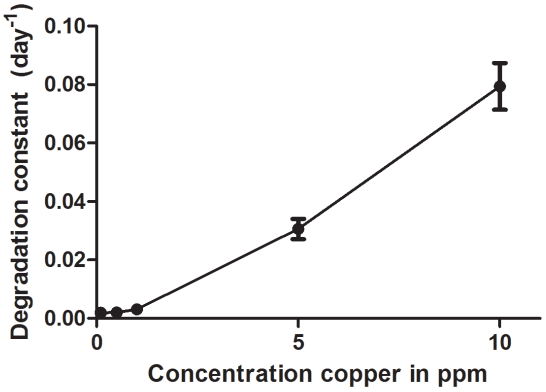

Figure 2. Degradation constants of triamcinolone acetonide in propylene glycol spiked with different concentrations of copper(II) acetate at 60 °C. First order degradation constants are shown (n=3 ± 95% CI).

A clear concentration dependence of the degradation constant can be observed. TCA degraded completely in the 10 ppm copper sample. In this sample TCA is present in a concentration which is 75 times higher than the copper molar concentration. This shows that significantly more TCA degraded than copper was present, indicating that copper catalyzed the reaction. Copper, or other trace metals as oxidation catalysts is further supported by literature (2,11,13). This indicates that TCA oxidation can be catalyzed and hence significantly increased even if only small traces of metal impurities are present.

The trace metal content in the excipients of the ointment

Limits for trace metals in topical products are set in the European Pharmacopoeia (Ph. Eur. general text 5.20). Limits for copper and iron are 250 and 1300 ppm respectively. Therefore, amounts <250 ppm are allowed to be present in lanolin, PG and petrolatum. Commonly suspected trace metals, copper, iron and nickel (11,13) were determined using ICP-OES in the individual excipients. Additionally, lanolin and petrolatum were extracted with PG and analyzed as well. **Table 2** shows the results.

Table 2. Average (± SD) trace metal content (ppm) detected in the individual ointment excipients and in lanolin-petrolatum extracted with propylene glycol (PG) (1:8:1) for one month at 60°C (n=4 for PG and petrolatum, n=3 for lanolin and n=1 PG extract).

Metal	PG	Lanolin	Petrolatum	PG extract
Iron (in ppm)	2.4 ± 1.5	11.9 ± 8.8	2.4 ± 1.7	5.6
Nickel (in ppm)	0.6 ± 0.3	1.5 ± 1.9	1.1 ± 1.1	0.8
Copper (in ppm)	0.5 ± 0.3	0.7 ± 0.3	0.8 ± 0.3	1.6

Trace metals content differed between excipients. It is clear that lanolin and petrolatum contain more trace metals than PG. Interestingly, the saturated PG extract shows a higher level of trace metal content, showing that trace metals transfer to PG when exposed to lanolin and petrolatum. This extract was free from any undissolved lanolin and petrolatum since at 60 °C the PG phase is phase separated from the lanolin-petrolatum mixture. Hence, it is to be expected that the metal content in the PG phase of the ointment increases when mixed with lanolin and petrolatum.

The influence of lanolin and petrolatum extracted with PG

As has been concluded above, TCA is predominantly present in the PG phase of the ointment. To check the influence of metals towards the TCA degradation, TCA was dissolved in PG and in lanolin and petrolatum extracted with PG. TCA content in this PG extract was determined after 16 days of storage at 60 °C and compared to untreated

PG. The remaining relative content was 85.2 ±0.5% and 97.2 ±0.04 % for the PG extract and in the untreated PG respectively. The faster degradation of TCA in the PG extract confirmed that the additional trace metals originating from the two excipients accelerate TCA degradation.

The degradation pathway

As described earlier, TCA degrades into the 21-aldehyde and the 17-carboxylic acid. To study how this degradation evolves, the ointment was stored at 60 °C and samples were analyzed at various time points for TCA, 21-aldehyde and 17-carboxylic acid content. **Fig. 3** presents the results.

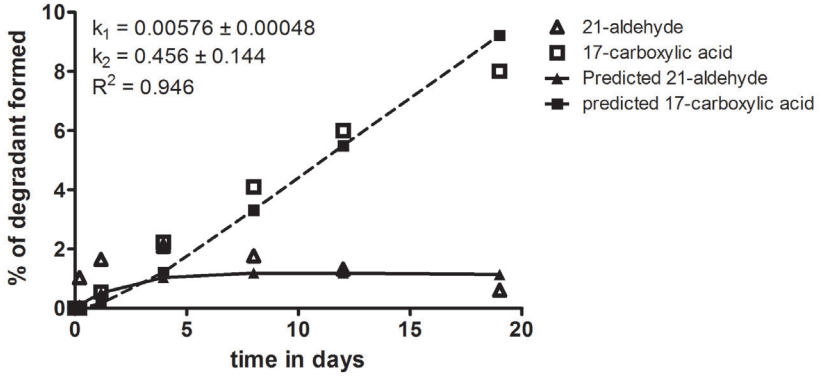

Figure 3. Formation of the two major degradation products of triamcinolone acetonide (TCA) in the ointment formulation. The predicted values are based on degradation kinetics models presented in (15). For the experimental data (n=3) a 95% CI was calculated, error bars however were too small to be visible.

Fig. 3 shows that the 21-aldehyde content increases initially and subsequently levels and even slightly decreases after 3 days. The 17-carboxylic acid forms after a lag time of a few hours and the content increases subsequently. This points in the direction of an reaction from A (TCA) to B (21-aldehyde) to C (17-carboxylic acid) (**Fig. 4**). This phenomenon has been described earlier by Waterman et al. (15). The degradation constants k1 and k2 can be calculated using Eq. 3 and 4.

$$\underset{[A]}{\text{TCA}} \xrightarrow{k_1} \underset{[B]}{\text{21-aldehyde}} \xrightarrow{k_2} \underset{[C]}{\text{17-carboxylic acid}}$$

Figure 4. Schematic overview of the TCA degradation route.

Equation 3: $$B_t = A_0 \left[\frac{k_1}{k_2 - k_1}\right][e^{-k_1 t} - e^{-k_2 t}]$$

Equation 4: $$C_t = A_0 \left(1 + \left[\frac{1}{k_1 - k_2}\right][k_2 e^{-k_1 t} - k_1 e^{-k_2 t}]\right)$$

Using these equations a degradation constant for the formation of the 21-aldehyde (k_1) was calculated to be 0.00576 ±0.00048 day^{-1} and for the formation of the 17-caboxylic acid (k_2) 0.456 ±0.144 day^{-1}. The fit for the equations was 0.946 further underlining the likelihood that the 17-carboxylic acid is formed from the 21-aldehyde in the ointment. Since $k_2 >> k_1$, more of the 17-carboxylic acid is present than 21-aldehyde shortly after the start of TCA degradation.

Based on the previously shown influence of trace metals and oxygen on the degradation of TCA, the following degradation mechanism can be proposed (**Fig. 5**).

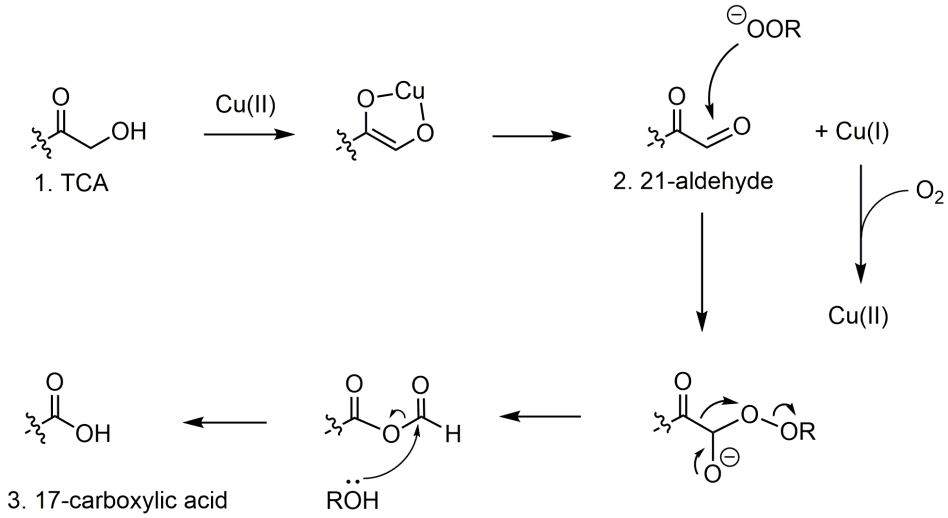

Figure 5. Proposed degradation mechanism of the C_{17} side chain of triamcinolone acetonide (TCA) (component 1) in TCA ointment FNA to 21-aldehyde (component 2) and 17-carboxyllic acid (component 3) (16,17).

Fig. 5 indicates that the suggested sequence of degradation as shown in **Fig. 4** is likely to occur according to the mechanism shown. The transformation of TCA to a 21-aldehyde is likely to involve Cu^{2+} to form both the 21-aldehyde and Cu^{+}, which subsequently forms Cu^{2+} using oxygen (16). The degradation of a 21-aldehyde to a 17-carboxylic acid has

been reported for a corticosteroid with the same 17-side chain as TCA, betamethasone, using LC-MS isotope experiments using $^{18}O_2$ as the oxidant (17). When the two described mechanisms are linked a water free transformation of TCA to a 21-aldehyde and a 17-carboxylic acid is proposed. Potentially, corticosteroids containing the same C_{17}-moiety as TCA may degrade likewise. This is further supported by literature on for example hydrocortisone showing similar degradation products formed (2).

The prevention of TCA degradation

Since the degradation of TCA depends on O_2 and trace metal content, the addition of a sacrificing antioxidant and a chelating agent could prevent degradation. To investigate the influence of such compounds, 0.1 % sodium metabisulfite (11) and 0.1 % 1,10-phenantroline as a chelating agent in organic environment (18) were dissolved in PG in combination with TCA and added to the lanolin-petrolatum mixture which was then stored at 60 °C for over 6 months in Erlenmeyer flasks. An ointment without additional ingredients was stored as well. **Fig. 6** presents the results.

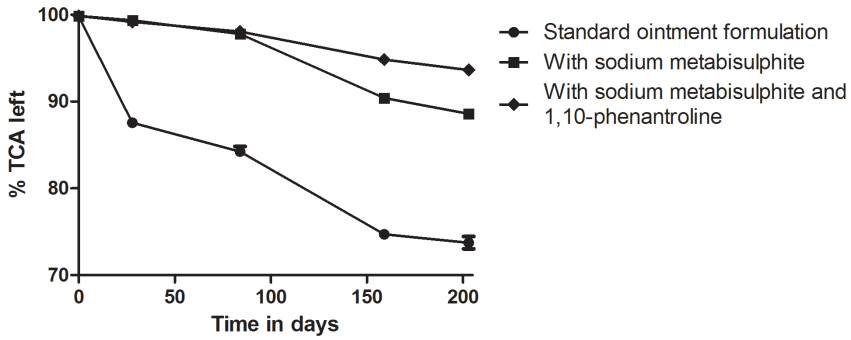

Figure 6. The influence of the addition of 0.1% sodium metabisulfite and a combination with 0.1% 1,10-phenantroline to TCA ointment FNA formulation. Samples were stored in closed glass containers at 60 ^{o}C, measurements conducted in n=3, 95% CI shown in error bars.

Fig. 6 clearly shows that sodium metabisulfite stabilizes TCA in the ointment showing that it can work as a sacrificing antioxidant in organic environment. The addition of 1,10-phenantroline shows an additional stabilizing effect. Logically, 1,10-phenantroline will sequester metals and thereby decrease the available amount of catalytic metals to react with TCA.

Conclusion

Oxidation of TCA takes place in the PG phase of the ointment. This oxidation is catalyzed by trace metals which are extracted from lanolin and petrolatum. An extensive degradation mechanism is proposed based on these findings. Sodium metabisulfite stabilized TCA in the ointment and 1,10-phenantroline shows an additional improvement when combined with sodium metabisulfite.

Acknowledgements

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Chapter 4.3

Topically used corticosteroids;

what is the big picture of drug product degradation?

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Abstract

Corticosteroids are widely used in topical formulations such as creams (aqueous) and ointments (non-aqueous). The generally used corticosteroids show large molecular resemblance, where especially the 20-keto-21-hydroxyl group bound to the 17 carbon is important for their chemical stability. Oxidation in both aqueous and non-aqueous environment occurs for triamcinolone acetonide (TCA), hydrocortisone (HC) and desoximethasone (DS). Besides the 20-keto-21-hydroxyl group, TCA, HC and DS have different other moieties attached to the same C17. These moieties are shown to influence not only the type of degradation product formed but also the degradation kinetics. Seven degradation products are found in total and a degradation mechanism is proposed. Furthermore, the transesterfication of betamethasone-17-valerate to betamethasone-21-valerate is shown to occur both in aqueous and non-aqueous environment. Finally, a comprehensive scheme of degradation pathways is presented that is applicable for both aqueous and non-aqueous formulations.

Introduction

Corticosteroids are anti-inflammatory agents of the steroid hormone class. Corticosteroids bind in the target cell to specific cytosolic glucocorticoid receptors and subsequently interact with glucocorticoid receptor response elements on DNA thereby altering gene expression (1). The affinity for the glucocorticoid receptor differs for each corticosteroid. Since the 1950's corticosteroids are used for many skin diseases, such as eczema and psoriasis. For these applications, corticosteroids are used in aqueous (creams and lotions) and in non-aqueous formulations (ointments).

One of the concerns with corticosteroid shelf life is the chemical stability. According to the ICH guideline, only limited amounts of degradation products may be present in the formulation (2). Furthermore, the identification of degradation products is important. Degradation can be studied using stress testing, which is described extensively elsewhere (3).

Corticosteroids are prone to oxidative degradation. This degradation predominantly occurs at the 17-side chain of corticosteroid molecules (4–7). Furthermore, degradation of the A ring (8–10) or hydrolysis of the acetonide moiety (11) has been described. A-ring degradation is a photochemical reaction and is considered irrelevant for most pharmaceutical formulations due to UV-protected packaging and is therefore not studied here. The 17-side chain generally consists of a 20-keto-21-hydroxyl group which is identical for the majority of corticosteroids. Nevertheless, several other possible side chains may be bound to the same 17-carbon atom, such as esters, hydroxyl and methyl groups. These extra side chain groups may result in altered potency and degradation mechanisms. The 20-keto-21-hydroxyl containing corticosteroids can be categorized into four groups, based on a different moiety on the 17-carbon atom, namely an acetonide, a hydrogen, a hydroxide or an ester (**Figure 1**).

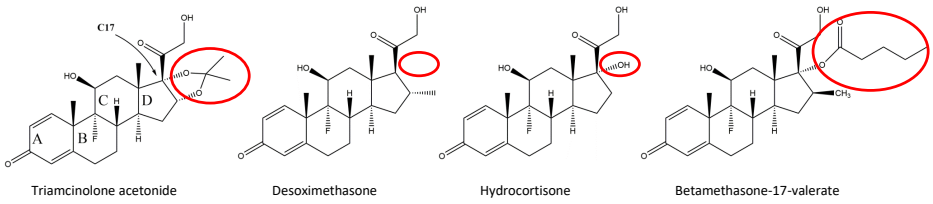
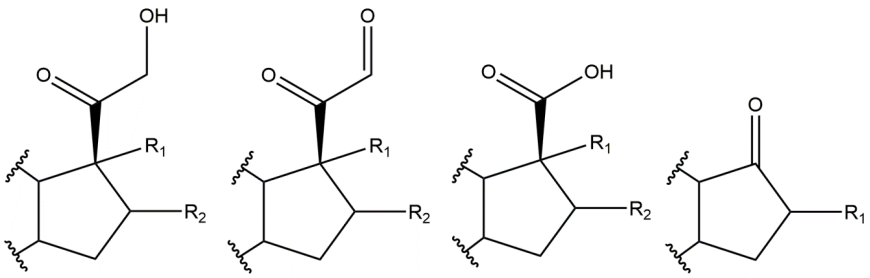


Figure 1. Overview of the different moieties that may be present on the 17-carbon atom in corticosteroids.

For all in **Fig. 1** mentioned corticosteroid groups a small selection of for topical application relevant degradation products is described in literature. For triamcinolone acetonide (TCA) (6,12) and hydrocortisone (HC) (4,13) a 17-carboxylic acid and 21-aldehyde have been reported and for desoximethasone (DS) only a 17-carboxylic acid (14). Specifically for HC a 17-ketone and a 17-carboxylic acid and 21-aldehyde without the 17-hydroxide moiety have been described (4,13). An overview of these degradation products is presented in **Fig. 2**. Betamethasone-17-valerate (B17V) can undergo transesterfication in acidic aqueous environment to betamethasone-21-valerate (B21V) which can subsequently form betamethasone through hydrolysis (15,16).



1. General corticosteroid 2. 21-aldehyde 3. 17-carboxylic acid 4. 17-ketone

Figure 2. The four degradation products of 20-keto-21-hydroxy corticosteroids that have been described in literature. R1 = OH, H or OR, R2 = H or OR.

In summary, only a limited amount of different degradation products have been described for 20-keto-21-hydroxyl corticosteroids. Unfortunately, an overview of the influence of chemical groups near this 17-side chain on the degradation is lacking. Furthermore, nearly all previously reported degradation studies were conducted in aqueous environment which does not necessarily apply to degradation in non-aqueous environment.

The aim of this study was to create an overview of the degradation pathways and kinetics of corticosteroid degradation in water and propylene glycol (PG). PG was chosen since it is the major non-aqueous solvent used in ointments, therefore it is considered a model for corticosteroids in non-aqueous environment (ointments).

Material and methods

Reagent and chemicals

The following chemicals were used: HPLC grade acetonitrile (ACN), dichloromethane, methanol (MeOH) and hexane (Avantor Performance, Center Vally, Pennsylvania, USA), copper(II) acetate (Alfa Aesar, Havehill, Massachusetts, USA), disodium edetate, hydrogen peroxide (H_2O_2), iron(III) chloride ($FeCl_3$), copper(II) chloride ($CuCl_2$) and tert-butyl peroxybenzoate (Merck, Darmstadt, Germany), propylene glycol (PG) (Brenntag, Dordrecht, The Netherlands), hydrocortisone (Sanofi Aventis, Buckhimham, UK), triamcinolone acetonide and betamethasone-17-valerate (Newchem, Milan, Italy) and desoximethasone (Farmabios, Pavia, Italy). 1 M hydrogen chloride (HCl) was prepared on site. Distilled, deionized water was prepared by a Elga Centra R 60/120 system (Woodridge, Illinois, USA).

Synthesis of the 21-aldehyde of TCA

The synthesis of the 21-aldehyde (compound 2, **Fig. 2**) was based on a method described in literature (5,12). The 21-aldehyde was synthesized by dissolving 600 mg TCA and 31.5 mg copper(II)acetate in 150 ml methanol. Air was bubbled through the solution for 60 minutes. The reaction was quenched by adding 20 ml of 2.5 mg/ml disodium edetate. The solution then was concentrated to 30 ml under cold air and extracted twice with 200 ml dichloromethane. The dichloromethane was evaporated under cold air. The formed 21-aldehyde was characterized using LC-MS.

Stress testing in water

Stress testing was performed on 0.05% solutions of the corticosteroid in a mixture of ACN:water (50:50). These solutions were exposed to the conditions described in **Table 1**. Storage temperature and time were chosen based on a degradation target of 5-20%. HCl and phosphate buffer at pH 9 were used to simulate acid and base catalyzed degradation. H_2O_2, $FeCl_3$ and $CuCl_2$ were used for peroxide and trace metal mediated oxidation respectively. Compounds were included in the results when their presence was in a concentration of ≥0.5%.

Stress testing in propylene glycol (PG)

The corticosteroids (0.05%) and reagents were dissolved in PG. For water free peroxide catalyzed degradation an organic peroxide, tertbutyl peroxybenzoate, was used (further referred to as 5% peroxide in PG). The other conditions were identical to the stress conditions in water (**Table 1**). Degradation constants (day^{-1}) were calculated assuming first

order kinetics and expressed as average ±%RSD. Experiments were conducted in duplicate and expressed as average (% RSD) except for 5% peroxide in PG, this was tested on a single sample.

Table 1. Stress conditions in water and PG

Water	Propylene glycol
0.1 M HCL (25°C)	
Phosphate buffer pH 9 (60 °C)	
5 mM $FeCl_3$ and $CuCl_2$ (40 °C)	5 mM $FeCl_3$ and $CuCl_2$ (40 °C)
3% H_2O_2 (25 °C)	Peroxide 5% (20 °C)

HPLC-UV

Chromatography was conducted on a Shimadzu Prominence-iLC-2030C 3D liquid chromatograph with diode array detector (Kyoto, Japan) and an Altima C18 RP18 column (250 x 4.6 mm^2, with 5 µm particles) (Mandel Scientific Company, Ontario, Canada). The flow rate was 1.5 ml/min and UV detection was at 241 nm. Mobile phase components were ACN with 20 mM phosphoric acid and water buffered at pH 2 using phosphoric acid. Injection volume was 20 µl. Chromatograms were obtained and analyzed with Shimadzu LabSolutions software version 5.5.7. For the four corticosteroids different gradient programs were run, these are shown in **Table 2**.

Table 2. Gradient programs run for triamcinolone acetonide (TCA), desoximethasone (DS), hydrocortisone (HC) and betamethasone-17-valerate (B17V). Percentage of acetonitrile with 20 mM phosphoric acid (ACN) that was used is shown, the rest is water buffered at pH 2 using phosphoric acid.

TCA		DS		HC		B17V	
Time (min)	% ACN	Time (min)	% ACN	Time (min)	% ACN	Time (min)	% ACN
0-12	0-32	0-10	25	0-15	25	0-10	25
12-30	32	10-27	25-45	15-32	25-45	10-27	25-45
30-40	32-70	27-40	45-75	32-45	45-75	27-40	45-75
40-42	70-0	40-41	75-25	45-46	75-25	40-41	75-25
42-47	0					41-46	25

LC-MS analysis

MS was conducted on a Micromass Quattro Ultima TQD system equipped with an electrospray ionization (ESI) source (Waters Chromatography, Etten-Leur, The Netherlands). Masses were scanned from m/z 50-1100, gas flow to 530 L/hr, gas temperature to 350 °C and voltage 3 kV. Data was analyzed with Masslynx version 4.0 software. The mobile phase components were ACN and water buffered at pH 2 using formic acid.

Results and discussion

Betamethasone-17-valerate (B17V) degradation

Corticosteroids containing an C17 ester moiety are known to degrade freely in aqueous environment (15,16). Degradation of these corticosteroids in non-aqueous environment has however not been described before. Therefore, this was studied in more detail, results are shown in **Table 3**.

Table 3. Degradation products identified using HPLC-MS for betamethasone 17-valerate (B17V). Amounts are expressed as relative percentage of total degradation, ±%RSD. PG = propyleneglycol.

Stress condition betamethasone 17 valerate				Amount of compound (expressed as relative % of total degradation, ±%RSD)			
Medium		Temp.	Time	% B17V	B21V	Betamethasone	Unknown
Water	0.1 N HCl	60 °C	7d	0.0 (0)	100.0 (0)		
	pH 9	60 °C	1d	50.0 (0.7)	2.0 (0)	48.0 (0.7)	
	3% H_2O_2	60 °C	7d	0.0 (0)	100.0 (0)		
	5 mM $FeCl_3$	40 °C	4d	0.0 (0)	100.0 (0)		
	5 mM $CuCl_2$	40 °C	5d	1.0 (6.7)	99.0 (0.1)		
PG	5% peroxide	60 °C	6d	2.0	98.0		
	5 mM $FeCl_3$	40 °C	7d	92.0 (0.1)	8.0 (0.9)		
	5 mM $CuCl_2$	60 °C	7d	84.0 (0.3)	16.0 (1.3)		4.0 (7.9)

As can be seen in **Table 3**, transesterfication occurred to a large extent under all conditions. This conversion has been described before for aqueous formulations (15,16), but not for non-aqueous environment. It is possible that the PG contained small amounts of water due to its hygroscopic nature. Another explanation can be the effect of PG or metal ions on transesterfication. **Fig. 3** proposes this degradation mechanism.

20-keto-21-hydroxyl corticosteroids are prone to oxidative degradation. However, the primary degradation product B21V did not undergo oxidative degradation under the conditions studied here. Thus, the ester moiety on the C21 apparently protects the compound against further oxidative degradation. Only after hydrolysis of the ester moiety to form betamethasone similar oxidative degradation as applies for HC will occur (17).

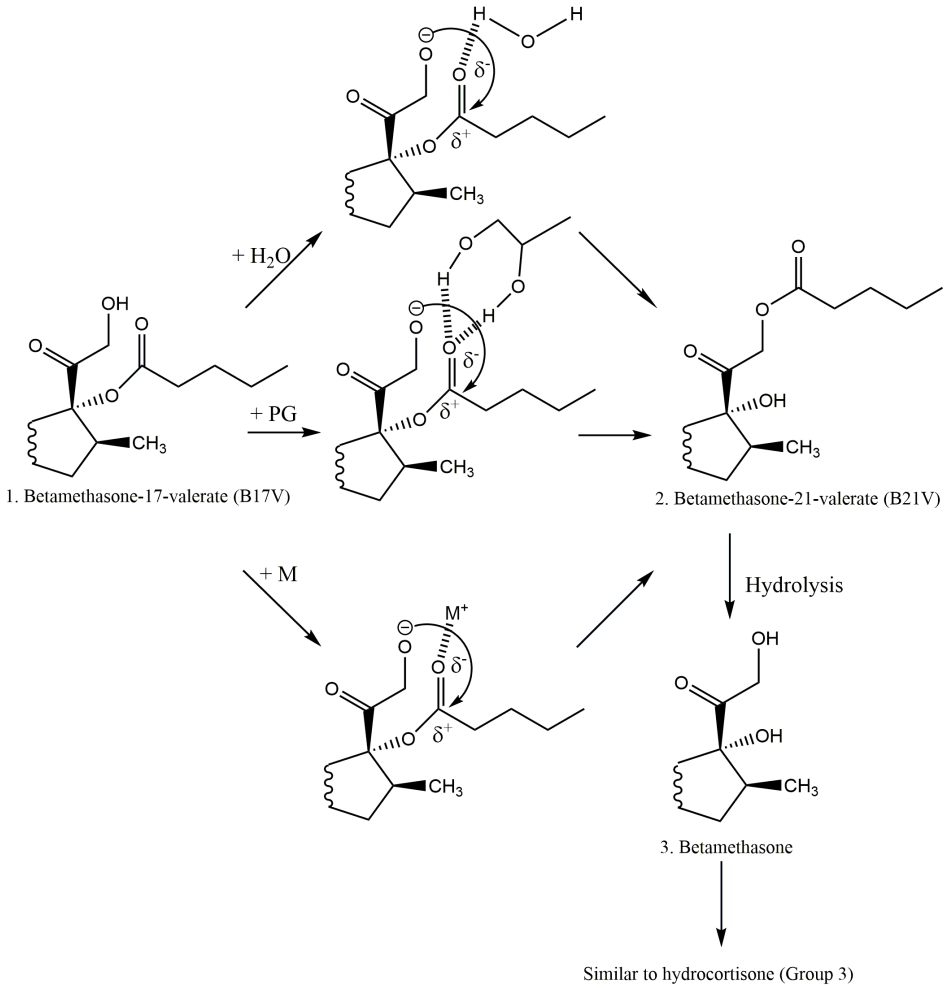


Figure 3. Proposed degradation mechanism of betamethasone-17-valerate (B17V) to betamethasone-21-valerate (B21V) and betamethasone in both aqueous and non polar environment.

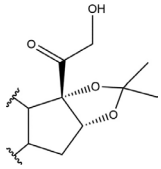
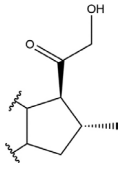
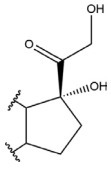
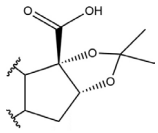
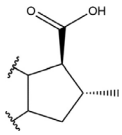
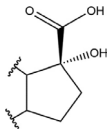
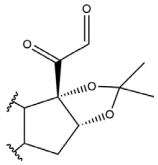
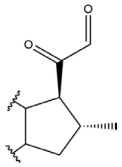
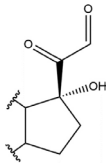
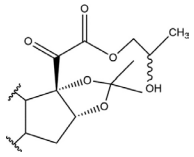
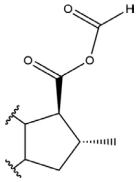
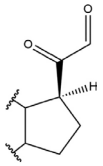
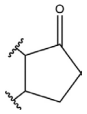
TCA, DS and HC degradation

Using mass spectrometry the m/z-ratio's of compounds in degradation study samples were studied. These were compared with m/z-ratio's of degradation products mentioned in literature. **Table 4** presents the degradation products that were identified in the samples.

Quantitative overview of the degradation products

In **Table 5** an overview is presented of the identity and relative amount of degradation product formed for TCA, HC and DS under oxidative stress conditions in water and PG.

Table 4. Degradation products identified using HPLC-MS for triamcinolone acetonide (TCA), desoximethasone (DS) and hydrocortisone (HC).

TCA	DS	HC
		
		
17-carboxylic acid of TCA (6,12) *m/z* = 420.4	17-carboxylic acid of DS (14) *m/z* = 362.5	17-carboxylic acid of HC (4) *m/z* = 348.5
		
21-aldehyde of TCA (6,12) *m/z* = 433.5	21-aldehyde-DS *m/z* = 376.5	21-aldehyde of HC (4) *m/z* = 361.5
		
21-glyoxilic PG ester (12) *m/z* = 507.5	Anhydride of DS *m/z* = 404.5	17-deoxy-21-alhyde of HC (4) *m/z* = 345.5
		
		17-ketone of HC (4) *m/z* = 319.5

The profiles of degradation products formed in aqueous and non-aqueous environment are similar among DS, HS and TCA. An 21-aldehyde and 17-carboxylic acid were found for HC, TCA and DS. However, clear differences in the degradation profile of the corticosteroids were also found. For example, HC was the only corticosteroid that degraded to compound 2 and 5; a 21-aldehyde lacking the C17-hydroxyl and a C17-ketone respectively, both compounds for which the C17-OH participates in the degradation mechanism. This is in accordance with literature (4,13). Furthermore, only TCA degraded to compound 6; a 21-glyoxilic PG ester which has been described in a study on a TCA ointment (12). Solely

Table 5. Overview of the degradation products formed in different stress conditions for triamcinolone acetonide (TCA), desoximethasone (DS) and hydrocortisone (HC). Amount of degradation product is expressed as relative percentage of total degradation.

Medium		Amount of compound (expressed as relative % of total degradation ±%RSD)		
		1. 21-aldehyde	2. 21-aldehyde without OH	3. 17-carboxylic acid
Water	0.1 N HCl	-	-	-
	pH 9	12.0 ± 0.0 (TCA) 14.0 ± 26.8 (HC)	20.0 ± 5.4 (HC)	24.0 ± 6.6 (TCA) 50.0 ± 4.7 (DS) 13.0 ± 0.0 (HC)
	3% H_2O_2	-	-	14.0 ± 0.0 (HC)
	5 mM $FeCl_3$	58.0 ± 0.15 (TCA) 10.0 ± 0.0 (HC)		34.0 ± 0.0 (DS) 15.0 ± 0.0 (HC)
	5 mM $CuCl_2$	100.0 ± 6.7 (TCA) 100.0 ± 0.0 (DS) 93.0 ± 0.0 (HC)	-	-
PG	5% peroxide	-	45.0 (HC)	100.0 (DS)
	5 mM $FeCl_3$	98.0 ± 1.4 (TCA)	-	16.0 ± 7.0 (DS)
	5 mM $CuCl_2$	91.0 ± 8.7 (TCA) 18.0 ± 6.6 (DS) 59.0 ± 15.5 (HC)	-	46.0 ± 1.3 (DS)

DS degraded to compound 4; an anhydride which has been described in literature as an intermediate (14). Interestingly, also a previously unreported 21-aldehyde was found for DS. Since the 21-aldehyde was also found for TCA and HC and reported in literature for other corticosteroids it seems logical that it will also form for DS. Furthermore, the 21-aldehyde was reported as precursor for the 17-carboxylic acid for betamethasone before (17).

Degradation profiles for corticosteroids under trace metal stress depended on the type of metal salt used. In water, the presence of copper led predominantly to the degradation into compound 1 (the 21-aldehyde) while the presence of iron resulted in a wider range of degradation products.

The data described above was combined with all relevant literature to propose an overview of 20-keto-21-hydroxylcorticosteroid degradation in general (4,12,14,17,18). **Fig. 4** presents this overview.

4. Anhydride of DS	5. 17-ketone	6. 21-glyoxilic PG ester	7. unknown rrt 21.5 min DS
-	-	-	-
	18.0 ± 21.1 (HC)	51.0 ± 1.5 (TCA)	49.0 ± 1.5 (DS)
-	86.0 ± 0.7 (HC)	-	-
64.0 ± 0.0 (DS)	76.0 ± 1.0 (HC)	42.0 ± 0.0 (TCA)	
-	-	-	-
	55.0 (HC)	-	-
84.0 ± 1.7 (DS)	100.0 ±2.1 (HC)	-	-
17.0 ± 0.0 (DS)	42.0 ± 8.6 (HC)	-	-

Degradation kinetics of corticosteroids in water and PG

HC, TCA and DS were exposed to identical degradation conditions and first-order degradation constants were calculated. **Table 6** presents these constants.

In **Table 6** it can be seen that degradation constants vary greatly dependent on the studied stress condition, indicating that the corticosteroids are not equally susceptible to every type of stress condition. Some results stand out. First, HC degraded faster than TCA and DS under aqueous stress conditions. Second, acidic and peroxide stress did not cause rapid degradation in any corticosteroid studied here. Third, aqueous alkaline environment and trace metals (especially iron) caused rapid degradation. This is more profound in PG than in water. Thus, both the solvent and the type of trace metal determine the degradation profile and the degradation rate as well.

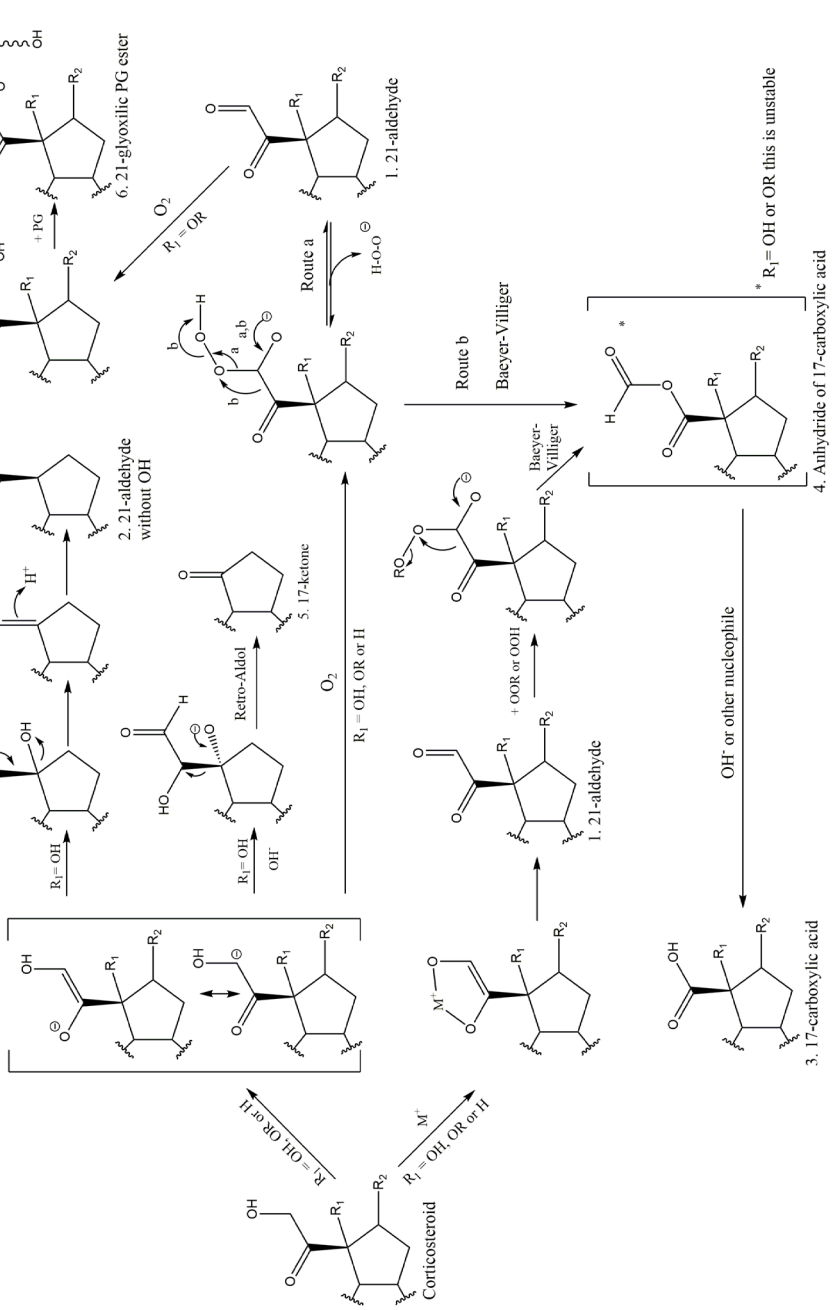


Figure 4. Proposed degradation mechanism for corticosteroids containing a 20-keto-21-hydroxyl side chain.

Table 6: Degradation constants of triamcinolone acetonide (TCA), hydrocortisone (HC) and desoximethasone (DS) in water and propylene glycol (PG) in the presence of different stress conditions (±%RSD).

Degradation constant (day^{-1})	TCA	HC	DS
In water			
0.1 N HCl (20 °C)	0.001 (0.0)	0.001 (0.0)	0.001 (0.0)
pH 9 (60 °C)	0.123 (±2.8)	0.100 (±0.4)	0.014 (±1.0)
3% H_2O_2 (20 °C)	0.001 (0.0)	0.018 (0.0)	0.001 (0.0)
5 mM $FeCl_3$ (40 °C)	0.002 (±7.9)	0.049 (±0.3)	0.022 (±1.0)
5 mM $CuCl_2$(40 °C)	0.005 (±15.1)	0.018 (±0.0)	0.001 (±0.0)
In propylene glycol			
5% peroxide (20 °C)	0.001	0.006	0.009
5 mM $FeCl_3$ (40 °C)	0.076 (±3.3)	0.068 (±2.3)	0.109 (±0.8)
5 mM $CuCl_2$ (40 °C)	0.007 (±37.5)	0.064 (±22.0)	0.011 (±2.0)

General discussion on corticosteroid degradation

From this study a number of conclusions can be drawn. First of all, it is clear that when an ester group is bound to the 17 carbon atom first transesterfication to a 21-ester and subsequently hydrolysis occurs. When no ester is present, oxidation is the predominant degradation mechanism. These corticosteroids form only a small selection of degradation products. A 17-carboxylic acid and a 21-aldehyde were found for all these corticosteroids. Interestingly, when a hydroxide group (HC) is bound to the 17 carbon atom a wider range of degradation products can be formed. Furthermore, for HC in general faster degradation was observed.

Metal salts show higher reactivity in non-aqueous environment compared to aqueous environment. $FeCl_3$ shows higher degradation constants compared to $CuCl_2$ and other degradation products are formed. Iron and copper are transition metals which can be involved in diverse chemical reactions. Copper has a more filled electron shell (i.e. d-shell) and hence is less reactive. This is why iron can bind six ligands whilst copper can only bind two ligands (19). Therefore, potentially more reactions with iron may occur compared to copper.

Interestingly, TCA shows a different degradation pattern compared to DS. TCA and DS both have a relatively non-reactive group bound to the 17-carbon (an acetonide and hydrogen respectively) in common. Therefore, it is remarkable that unique degradation products were found for both TCA and DS such as the 21-glyoxylic PG ester for TCA and an anhydride for DS. Also a not identified degradation product was found for DS in alkaline conditions. Apparently, the acetonide and hydrogen group influence corticosteroid degradation. Potentially, this can be explained by the fact that an acetonide is more electron rich which may influence the degradation.

Conclusion

An overview of the quantitative and qualitative degradation of a wide range of corticosteroids is presented in both aqueous and non-aqueous environment for the first time. Corticosteroids containing a 20-keto-21-hydroxyl group bound to the 17 carbon atom degrade predominantly by oxidation. The other group (i.e. a hydrogen, hydroxide or acetonide) bound to the 17 carbon atom not only influences degradation kinetics but also the type of degradation product formed. When an ester group is bound to the 17 carbon first transesterfication and hydrolysis occur before it further degrades by oxidation. By using this overview degradation of corticosteroids can be predicted and potentially inhibited through the understanding of the degradation mechanisms presented here.

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Chapter 5

General Discussion

General Discussion

The majority of the research on the fundamental physical characterization of dermatological products and excipients was conducted before the 1980's. Since then, the arsenal of analytical techniques has been significantly improved. Despite this increase in investigational possibilities, there is hardly any recent innovation in dermatological products. Furthermore, authorities increasingly demand the industry to apply Quality by Design, besides increased requirements on chemical drug degradation currently exist. The consequence of these three developments is that a higher degree of product and process understanding is demanded for dermatological products.

This dissertation aimed to create a deeper understanding of a widely used ointment excipient, processing and scale up and stability of corticosteroids in ointments.

Critical material attributes (CMAs)

In chapter 2 the CMAs of the widely used excipient petrolatum are studied. Petrolatum, or white soft paraffin, is a viscoelastic material. This means that it combines both solid- and liquid-like properties. Its rheological properties are known to be complex (1,2). In the past, this complex rheological behavior has been attributed to a three dimensional crystalline network which shows a resistance to flow (2–4). However, no convincing evidence of such a network exists. Therefore, the petrolatum structure was studied using powder X-ray diffractometry and rheometry in chapter 2.1. In this chapter it is concluded that the label "Ph. Eur. quality" does not correspond to a constant consistency. Different Ph. Eur. quality petrolatum grades were rheologically characterized and significant differences in yield stress were observed. Furthermore, thermal history was shown to significantly influence petrolatum consistency. Fast cooling results in more rigid petrolatum compared to slowly cooling. The influence of thermal history on the structure can possibly be explained by small differences in crystallinity dependent on cooling rate. Petrolatum was shown to contain small fractions of crystalline material e.g. <1.5%. These crystals are small, approximately 20-50 nm in size. Because of this size, small differences in crystalline mass account for large differences in number of crystals. Potentially, these cause the observed differences in petrolatum structure. This is studied in more detail in chapter 2.2 where synchrotron small- and wide-angle X-ray scattering, pulsed NMR, microscopy and rheometry were used to elucidate the petrolatum structure on nano-, micro- and macrometer scale. The combination of these techniques shows that petrolatum is composed of 21 % solid material at room temperature. This

consists of partly crystalline lamellar sheets which are packed in stacks. The occurrence of these lamellar sheets is temperature dependent and the number of lamellar stacks is dependent on thermal history. It was shown that rheological differences in petrolatum can be explained by the number of lamellar stacks present, where more lamellar stacks result in more rigid petrolatum. These lamellar sheets are partly crystalline. It is shown that these crystals form domains of approximately 50 nm and consist of rhombohedral unit cells. Interestingly, the amount of crystalline material appeared not to depend on thermal history as was proposed in chapter 2.1. Furthermore, the in chapter 2.1 mentioned value of 1.5% seems to be considerably higher, namely ~8%. This difference may be caused by differences in integration method of the WAXS data and/or due to the lower accuracy of the powder X-ray diffractometer used in chapter 2.1 compared to the synchrotron WAXS used in chapter 2.2.

Based on these results the petrolatum structure can be described on the < nm dimensions as rhombohedral crystal unit cells, which compose part of the lamellar sheets found on the nm - µm scale. Stacks of these lamellar sheets form a three dimensional network entrapping the liquid fraction and provide the macrometer structure of petrolatum resulting in its viscoelastic properties. Therefore, a slight paradigm shift from a crystalline network to a partly crystalline network of lamellar stacks is proposed for petrolatum. Knowledge of the variable nature of petrolatum and the more thorough understanding of the structure of petrolatum can help in optimizing both formulation and production processes.

Critical Process Parameters (CPPs)

In chapter 3 the processing and upscaling of cetomacrogol ointment are studied. In general, a production process involves a multitude of process steps and process variables. Every single parameter may affect the properties of the final product and therefore it is necessary to have knowledge about critical settings. In the production process of ointments multiple parameters can be varied simultaneously. For example; when the cooling rate is increased, the total production time will be reduced and therefore less mixing takes place. Both the cooling rate and the degree of mixing may be of importance with respect to the product's homogeneity, consistency etcetera. Therefore, a design of experiments (DoE) approach can be a great tool to study a production process. A DoE is a statistical tool to define the impact of variables in a multi-variable process. Recently, a novel type of DoE was developed, the definitive screening design (DSD) (5). This type of DoE has two significant advances over the more traditional factorial DoE designs. In traditional designs variables are usually studied on two levels, meaning that

when a variable shows a non-linear effect this cannot be detected. In a DSD variables are studied on three levels, therefore non-linear effects can be detected. Furthermore, less experiments have to be conducted compared to traditional designs. In chapter 3.1 this DSD was applied on the lab scale production process of cetomacrogol ointment. The consistency of cetomacrogol ointment, expressed as yield stress, was shown to be highly dependent on process settings. Mixing rate and container filling temperature were the two critical process parameters (CPPs). The consistency is thought to reflect structural differences of the cetomacrogol ointment. When this is compared with the findings of chapter 2.2 on the lamellar structure of petrolatum, it is likely that cetomacrogol ointment, which also contains petrolatum, exhibits structure dependency upon processing. It can be hypothesised what may cause the change in rheological properties. For example, the two solid excipients in the ointment; cetomacrogol wax and petrolatum are chemically different. Cetomacrogol wax is relatively hydrophilic whilst petrolatum is hydrophobic. Therefore, these two components will most likely form two separate phases. The petrolatum will form a lamellar structure and cetomacrogolwax a currently unknown structure. These two phases may influence the rheological properties on a macrometer scale by friction between the structures when moved.

This lab scale process understanding contributed substantially to the effective scale up of this process to industrial scale. The scale up of a production process is a major challenge in pharmaceutical industry. In chapter 3.2 it is shown that with lab scale process understanding of the CPPs, the industrial scale experiments could focus only on these CPPs. In contrast to the small scale, mixing rate was not found to effect product quality on industrial scale. Logically, this can be due to the different mixing principles used. Tube filling temperature was shown to be critical on industrial scale. It was found to be highly affected by product viscosity. Therefore, on the industrial scale experiments were conducted to establish a process window for product viscosity to successfully fill product in tubes. This approach was subsequently verified for a number of products (both creams and ointments) and showed general applicability. This shows that the relatively pragmatic approach used in chapter 3.2 can be successful in scaling up a production process. Another approach would be to adopt the similarity principle, which assumes that across all equipment and process scales equal ratios between for example dimensions, forces and temperature gradients should be achieved (6). Here, often dimensionless numbers are used as expression of these ratios. In practice, the requirement of similarity is impossible to fully meet (7). Therefore, dimensional analysis can be complex and time consuming.

Critical Quality Attributes (CQAs)

In chapter 4 the CQAs are studied for triamcinolone acetonide (TCA) in an ointment formulation ("TCA zalf FNA"). In chapter 4.1 the development and validation of a stability indicating HPLC-UV method for the detection of all degradation products of TCA in "TCA zalf FNA" is described. Interestingly, in this study an extensive set of oxidative stress tests was used to predict degradation product formation in the ointment during storage. It was shown that only by including unconventional stress tests such as azobisisobutyronitrile (AIBN) and both iron and copper salts a complete picture of degradation product formation was acquired. This indicates that in product development a wider set of stress tests should be used than are conventionally used. This method was subsequently used on the ointment formulation to gain insight into the degradation mechanism (chapter 4.2). As has been stated previously, TCA degradation is an oxidative process. In chapter 4.2 the role of the ointment excipients on TCA oxidation is described. It is shown that the excipients lanolin and petrolatum influence TCA degradation significantly. Lanolin and petrolatum contain small traces (few ppm) of metals such as copper, iron and nickel. These metals were shown to transfer to the propyleneglycol phase in the ointment formulation. This is the phase in which TCA resides and the increase in trace metal content showed a significant increase in TCA degradation. Trace metals may catalyze oxidation reactions and by using copper acetate as model for the trace metals in the excipients a copper concentration dependent increase in degradation constant for TCA was found. Furthermore, the degradation pathway was elucidated; the alcohol group in the 20-keto-21-hydroxyl sidechain of TCA first oxidizes to an aldehyde. This compound subsequently degrades to four other degradation products. The formation of the aldehyde was shown to be catalyzed by metals. Since the 20-keto-21-hydroxyl side chain is not unique for TCA but rather common for corticosteroids, the findings of chapter 4.2 were applied to hydrocortisone (HC), desoximethasone (DS), TCA and bethamethasone-17-valerate (B17V). These findings are described in chapter 4.3. Here, the degradation of the aforementioned corticosteroids in aqueous and non-aqueous environment is described. HC, DS and TCA all have the 20-keto-21-hydroxyl group bound to the C_{17} in common. Nevertheless, several other groups may be bound to this C_{17}. For example, a hydroxide for HC, a hydrogen for DS and an acetonide for TCA. It was shown that this group influences both the qualitative and the quantitative degradation of these corticosteroids. In general, seven degradation products are formed for these corticosteroids; especially HC was shown to degrade into the largest variety of degradation products (five in total) and generally showed the highest degradation constants. Furthermore, the transesterfication of B17V to betamethasone-21-valerate was shown to occur both in

aqueous and non-aqueous environment. Finally, a comprehensive scheme of degradation pathways is shown that is applicable for both aqueous and non-aqueous formulations. These three chapters provide insight into corticosteroid degradation in ointments. These non-aqueous vehicles are usually not included in published degradation studies of pharmaceuticals. Especially the fact that oxidation occurs in non-aqueous environment and can be catalyzed by trace metals and inhibited by the addition of the antioxidant sodium metabisulphite, which is normally only used in aqueous environment, will most likely improve the stability of oxidation sensitive drugs in ointments in the future.

Future perspectives and concluding remarks

In summary, this dissertation demonstrates that product and process quality may dependent on both excipient characteristics, formulation and production processes. By including a systematical study of all three in the product and process development of dermatological products, significant improvements in product quality can be expected. Tools such as design of experiments (DoE) and especially the novel definitive screening design (DSD) will ease the necessary experimentation to acquire the product and process understanding. Furthermore, despite the fact that ointment formulations are generally "old", due to the recent advances in analytical techniques and demands from authorities on product quality, especially today improvements in ointment formulations should be expected.

More specifically, this dissertation outlines the variable rheological properties of petrolatum and shows that these varieties are caused by changed in the ordering of the lamellar sheets in stacks. These results can be used in the (re)development of ointment and cream formulations containing petrolatum. Furthermore, it is likely that the methodology used to characterize petrolatum can be used for other excipients and formulations likewise.Furthermore, based on the results for petrolatum a new definition for ointments can be proposed. Defining ointments as "single phase" is not even suitable for defining petrolatum. Therefore ointments should be defined as "non-aqueous, multiphase systems in which solids and miscible liquids may be dispersed", this definition will allow proper categorization of ointments and creams in the future.

Scale up will remain a major challenge in pharmaceutical industry, however process understanding on lab scale batches can reduce the amount of effort needed to successfully scale up a production process.

For the chemical degradation of corticosteroids in ointment formulations it was always assumed that the “TCA zalf FNA” formulation, which has been on the market for several decades, was a chemically stable product. Furthermore, the analytical method described in the British Pharmacopoeia to analyze the degradation of TCA in this ointment was assumed suitable. For both these assumptions it is now clear that especially for these “old” formulations and methods innovations should be demanded today. This dissertation shows that by challenging established products and methodologies, through the adaptation of new techniques and methodologies significant improvements can be made. The next step in the optimization of corticosteroid formulations will be to use the insights provided in this dissertation on the degradation mechanisms to formulate more stable products.

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Appendix

Summary

Nederlandse samenvatting

List of coauthors,

List of publications

Publicaties in populaire “high-impact” journals

Dankwoord,

Curriculum vitae

Appendix A.1

Summary

Summary

The majority of the research on dermatological products and excipients was conducted before the 1980's. Since then, the analytical techniques have significantly improved. Despite this increase in investigational possibilities, there is hardly any recent innovation in dermatological products. It is expected that a more thorough understanding of the physical properties of excipients and formulations will eventually lead to dermatological product innovation.

Furthermore, in the past decades a paradigm shift has taken place within the pharmaceutical industry from a one-factor-at-a-time experimentation strategy to a more comprehensive, statistical and science based approach towards product development: Quality by Design (QbD). This applies to the production of dermatological products as well but has not yet been fully implemented in the pharmaceutical industry.

Additionally, the requirements of authorities on the degree of chemical drug degradation have increased. As a consequence, more understanding of the chemical degradation processes in dermatological product is needed.

The consequence of all the above described developments is that a higher degree of product and process understanding is demanded for dermatological products. This requires more thorough biopharmaceutical research to be conducted on these products and production processes.

This dissertation aims to acquire the necessary product and process knowledge of ointments.

One way to study this would be to use the Quality by Design (QbD) paradigm. In this way a systematical study of different formulation, process and quality attributes is conducted. For the formulation, Critical Material Attributes (CMAs) of the individual excipients are defined. In the production process the Critical Process Parameters (CPPs) are identified and for the end product the Critical Quality Attributes (CQAs) can be studied.

Critical Material Attributes (CMAs)

For one of the major ointment excipients, petrolatum (or Vaseline®), the CMAs were studied in chapter 2. One CMA for petrolatum is its consistency. One way to determine

the consistency is by evaluating the rheological properties. Of these properties, the spreadability is of major importance.

In chapter 2.1 petrolatum was characterized using powder X-ray diffractometry and rheometry. Large differences in spreadability between commercially available Ph. Eur. quality petrolatum were found. These differences not only depend on composition (e.g. different batches or grades) but also on thermal history. Rapid cooling leads to a more rigid petrolatum product. This is suggested to be caused by small differences in crystallinity. The rapidly cooled petrolatum resulted in 1.5% crystalline material compared to 0.7% for the slowly cooled petrolatum. These crystals are approximately 20-50 nm in size, as determined by powder X-ray diffratometry. Due to this small size, a slight increase in crystallinity accounts for a large increase in the number of crystals. It is thought that large numbers of crystals can have a substantial effect on the rheological properties. This was demonstrated using small (15 nm) colloidal silicon dioxide particles as model "crystals". These findings were studied in more detail in chapter 2.2. Synchrotron small- and wide-angle X-ray scattering, pulsed NMR, microscopy and rheometry were used to elucidate the petrolatum structure on nano-, micro- and macrometer scale. The combination of these techniques shows that petrolatum is composed of 21 % solid material at room temperature. This solid fraction consists of lamellar sheets which are packed in stacks. These lamellar sheets are formed during cooling. The rheological differences in petrolatum can be explained by the number of lamellar stacks present. When more lamellar stacks are present, the petrolatum is more rigid.

Using wide-angle X-ray scattering (WAXS) it was shown that the lamellar sheets are partly crystalline. These crystals form domains of approximately 50 nm and consist of rhombohedral unit cells. Interestingly, this time no differences in crystallinity were found dependent on thermal history. This is in contrast to the results of chapter 2.1. This difference may be caused by differences in integration method of the WAXS data and/or due to the lower accuracy of the powder X-ray diffractometer used in chapter 2.1 compared to the synchrotron WAXS used in chapter 2.2. Based on these results the petrolatum structure can be described on the < nm dimensions as rhombohedral crystal unit cells. On the micrometer range petrolatum is composed of lamellar sheets. On the macrometer scale, stacks of these lamellar sheets form a three dimensional network entrapping the liquid fraction. These structures are responsible for the viscoelastic properties of petrolatum.

Critical Process Parameters (CPPs)

In chapter 3 the CPPs are studied for cetomacrogol ointment. The influence of individual variables in the production process on ointment yield stress, a measure for spreadability, was studied. A design of experiments (DoE) approach was used. Using DoE, a production process can be systematically studied using statistical tools to reduce the amount of experiments needed. A novel and more powerful type of DoE, namely a Definitive Screening Design (DSD) was used. The influence of production parameters such as heating temperature, cooling rate, mixing rate and container filling temperature was studied. First of all, it was shown that by varying parameters in the production process substantial differences in the yield stress of the ointment were observed. Only 5 of the 14 produced batches were within the pre-defined requirements for yield stress. These requirements are based on the outcomes of an internal test panel (n=10). Using the DSD statistics it was found that mixing rate and filling temperature significantly influence ointment yield stress ($p = 0.0013$ and 0.0065 respectively). The outcomes of the DSD study are described in a mathematical function which can predict ointment yield stress based on the settings for all studied variables. The certainty of this calculated "model" is 97.2%. This was verified in triplicate for batches produced under the optimal conditions. Yield stresses for these batches showed a marginal deviation of the predicted value of -2.8%. The outcomes of this study were subsequently evaluated on industrial production scale in chapter 3.2. Upscaling is a major challenge in pharmaceutical industry. One way to approach the upscaling of a process is to acquire process understanding on a lab scale. Once it is known what parameters significantly determine the properties of the product, upscaling can be performed with these so called "critical" parameters. Therefore, only the influence of the previously found critical parameters mixing rate and filling temperature was evaluated on industrial scale production. Interestingly, on industrial scale production the mixing rate did not affect product quality. Mixing is greatly dependent on the mixing principle and dimensions of the manufacturing equipment. For the industrial scale a mixing-homogenizer combination was operated whilst on lab scale production only a small mixing vessel without an homogenizer was applied. Especially the homogenizer will most likely influence ointment structure on colloidal dimensions due to the high shear forces. Furthermore, important dimensionless numbers (Froude and Reynolds), which indicate the geometrical similarity for production scales, are different. Therefore, the lab and industrial manufacturing equipment are not comparable, this may cause the differences in results.

Filling temperature on the other hand did show a critical influence on ointment yield stress on industrial scale. This is similar to the lab scale results. Therefore, the influence of filling temperature on ointment yield stress appears independent of the manufacturing scale or equipment. The filling process on the other hand is more challenging on industrial scale compared to lab scale. Usually, on a lab scale only a few containers are filled after production, whilst on industrial scale automated filling machines are applied. This process is largely dependent on the rheological properties of the product, or viscosity. For this viscosity a process window for successfully filling of product was established. The viscosity of an ointment or cream is highly temperature dependent. Therefore, by using the relationship between temperature and viscosity, the range of filling temperatures can be determined. For cetomacrogol ointment, it was shown that between 26.4 and 33.0 ^{o}C tubes can be filled with minimal weight variation (<0.5 %). At lower or higher temperatures it appeared impossible to operate the filling machine at the desired rate of 75 tubes/min. The process window for product viscosity was subsequently applied to other products (both creams and ointments) and showed general applicability. Based on these results, it can be concluded that the upscaling up of a production process can be based on lab scale process understanding.

Critical Quality Attributes (CQAs)

In chapter 4 the CQAs were studied for triamcinolone acetonide (TCA) in an ointment formulation (TCA zalf FNA (Formularium Nederlandse Apothekers)). In chapter 4.1 the development and validation of a stability indicating HPLC-UV method for the detection of all degradation products of TCA in "TCA zalf FNA" is described. The method was validated according to the requirements of the ICH guidelines. Degradation of TCA is an oxidative process. Interestingly, in this study an extensive set of oxidative stress tests was used to predict degradation product formation in the ointment during storage. It was shown that by including unconventional stress tests such as azobisisobutyronitrile (AIBN) and both iron and copper salts a complete picture of degradation product formation was acquired. This was subsequently verified using a four and a half year stored ointment sample. This method was subsequently used on the ointment formulation to gain insight into the degradation mechanism. In chapter 4.2 the role of the ointment excipients on TCA oxidation is described. Interestingly, it is shown that the excipients lanolin and petrolatum influence TCA degradation significantly. Using inductive coupled plasma optical emission spectrometry (ICP-OES) it was shown that lanolin and petrolatum contain small traces (few ppm) of metals such as copper, iron and nickel. These metals were shown to accumulate in the propyleneglycol phase in the ointment formulation.

This is the phase in which TCA resides. The increase in trace metal content causes a significant increase in TCA degradation. Trace metals are known to catalyze oxidation reactions. Copper acetate was used as a model for the trace metals in the excipients. The degradation constant for TCA appeared to increase upon exposure to higher copper concentrations. Furthermore, the degradation pathway for TCA was elucidated. The alcohol group in the 20-keto-21-hydroxyl sidechain of TCA first oxidizes to an aldehyde. This compound subsequently degrades to four other degradation products. The formation of the aldehyde was shown to be catalyzed by metals. Interestingly, the 20-keto-21-hydroxyl side chain is not unique for TCA but rather common for corticosteroids. The findings of chapter 4.2 were generalized using hydrocortisone (HC), desoximethasone (DS), TCA and bethamethasone-17-valerate (B17V) and described in chapter 4.3. What started off as a review of all available literature on corticosteroid degradation evolved in an experimental chapter on the degradation of the aforementioned corticosteroids in aqueous and non-aqueous environment due to the lack of sufficient published data. HC, DS and TCA all have the C_{17} bound 20-keto-21-hydroxyl group in common. However, in addition to the 20-keto-21-hydroxyl group, different groups are bound to this C_{17} atom for HC, DS and TCA. It was shown that this group influences both the qualitative and the quantitative degradation of these corticosteroids. In general, seven degradation products are formed for these corticosteroids. Especially HC was shown to degrade into the largest variety of degradation products (five in total) and generally showed the highest degradation constants. Furthermore, the transesterfication of B17V to betamethasone-21-valerate was shown to occur both in aqueous and non-aqueous environment. Finally, a comprehensive scheme of degradation pathways is shown that is applicable for both aqueous and non-aqueous formulations.

In summary, this dissertation demonstrates that product and process quality may dependent on both excipient characteristics, formulation and production process. By using a QbD approach the necessary product and process understanding can be acquired. The outcomes highlighted in this dissertation show that future product related research should focus even more on the influence of excipients, process and formulation on critical product characteristics.

Appendix A.2

Nederlandse samenvatting

Begrippenlijst en (belangrijke) afkortingen:

B17V:	Betamethason-17-valeraat
CMAs:	Critical Material Attributes
CPPs:	Critical Process Parameters
CQAs:	Critical Quality Attributes
DoE:	Design of Experiments
DS:	Desoximetason
DSD:	Definitive Screening Design
HC:	Hydrocortison
Kristal:	Periodiek (in een rooster) gerangschikte moleculen of atomen
QbD:	Quality by Design
Rheologie:	Het bestuderen van stromingseigenschappen van materialen
Rheometrie:	Het meten van rheologische eigenschappen
SAXS:	Small angle X-ray scattering
TCA:	Triamcinolon acetonide
WAXS:	Wide angle X-ray scattering
X-ray diffractometry:	Een techniek om aan de hand van de verstrooiing van röntgenstralen (X-ray) de structuur van vaste stoffen te bepalen.

Nederlandse samenvatting

Er wordt veel onderzoek gedaan aan geneesmiddelen. Nieuwe ontwikkelingen op het gebied van biotechnologie, immunologie en genetica staan volop in de belangstelling. Dit in tegenstelling tot reeds bestaande producten. Met name op het gebied van geneesmiddelen voor de huid is naar verhouding beperkte aandacht. Het grootste gedeelte van het onderzoek naar dit soort dermatologische producten (zalven, crèmes, gelen, etcetera) vond voor de jaren 80 van de vorige eeuw plaats. Sinds die tijd zijn de methoden om de kwaliteit van deze producten te onderzoek sterk verbeterd. Ondanks deze ontwikkelingen is er maar zeer beperkt sprake van innovatie van dermatologische preparaten. Het is waarschijnlijk dat een meer fundamenteel begrip van de fysische en chemische eigenschappen van grondstoffen en formuleringen zullen leiden tot het verbeteren van zalven en crèmes.

Daarnaast heeft in de laatste decennia een verschuiving plaatsgevonden binnen de farmaceutische industrie. Hierbij is de strategie van “trial-and-error” naar een meer statistisch en wetenschappelijk onderbouwde benadering verschoven, namelijk: Quality by Design. Dit zou ook toepasbaar moeten zijn voor de ontwikkeling van dermatologische preparaten.

Ten slotte zijn de eisen, die door de autoriteiten aan de mate van chemische product ontleding gesteld worden, sterk toegenomen. Daarom is een beter begrip van de ontledingsprocessen in dermatologische producten noodzakelijk.

De consequentie van deze ontwikkelingen is dat er een toenemende behoefte is aan kennis over het product en het bijbehorende productie proces is.

Dit proefschrift tracht de noodzakelijke product- en proceskennis over dermatologische producten, in het specifiek zalven, te vergroten.

Een manier om dit te bewerkstelligen is door een zogenoemde Quality by Design (QbD) benadering toe te passen. Hiermee wordt systematisch bestudeerd welke eigenschappen van de formulering, proces en eindproduct belangrijk zijn voor de kwaliteit van het product. Voor de formulering worden zogenoemde Critical Material Attributes (CMAs) van de verschillende afzonderlijke grondstoffen gedefinieerd. De kritische variabelen in het productieproces worden Critical Process Parameters (CPPs) genoemd. De

eigenschappen die kritisch zijn voor de kwaliteit van het eindproduct worden Critical Quality Attributes (CQAs) genoemd.

Critical Material Attributes (CMAs)

Van één van de belangrijkste grondstoffen voor zalven, vaseline, worden de CMAs beschreven in hoofdstuk 2. Eén van de CMAs is de consistentie van vaseline. Deze kan bestudeerd worden door middel van rheometrie. Hiermee kan bijvoorbeeld de smeerbaarheid van een product bestudeerd worden. Dit is vanzelfsprekend een belangrijke producteigenschap voor het uiteindelijk gebruik door de patiënt. In hoofdstuk 2.1 staat beschreven hoe de structuur van vaseline gekarakteriseerd is door X-ray diffractometrie en rheometrie. Er blijken grote verschillen in smeerbaarheid tussen verschillende commercieel verkrijgbare vaselines te bestaan. Deze verschillen kunnen niet alleen verklaard worden door een verschil in samenstelling tussen de verschillende producten, maar ook door verschillen in de zogenaamde thermische historie. Bijvoorbeeld, als vaseline snel afkoelt tijdens productie, dan leidt dit tot een moeilijker uit te smeren (meer rigide) product. Een mogelijke verklaring hiervoor kan zijn dat er kleine verschillen in kristalliniteit bestaan tussen vaselines met verschillende thermische historie. Uit meting met X-ray diffractometrie bleek een snel afgekoelde vaseline bleek 1,5% uit kristallen te bestaan en een langzaam afgekoelde vaseline uit 0,7%. Deze kristallen zijn zeer klein, circa 20 – 50 nanometer (nm). Doordat ze zo klein zijn, kan een minimale verhoging in percentage kristalliniteit in een sterke verhoging van het aantal kristallen resulteren. Het lijkt aannemelijk dat een sterke groei in het aantal kristallen een belangrijke invloed kan hebben op de rigiditeit van vaseline. Om dit te demonstreren is de modelstof colloïdaal silicium dioxide, met een primaire deeltjesgrootte van 15 nm, gekozen vanwege zijn vergelijkbare deeltjesgrootte met de kristallen in vaseline. Bij toevoeging van slechts kleine hoeveelheden van dit materiaal bleek de rigiditeit van vaseline sterk toe te nemen. Dit impliceert dat een kleine toename in hoeveelheid kristallijn materiaal een significante invloed op de rigiditeit kan hebben.
Om de structuur van vaseline, en de invloed van thermische historie in het bijzonder, verder te bestuderen is een aantal additionele technieken toegepast. De resultaten hiervan staan beschreven in hoofdstuk 2.2. De gebruikte technieken zijn: synchrotron small- en wide-angle X-ray scattering (SAXS en WAXS respectievelijk), pulsed NMR, microscopie en rheometrie. De combinatie van deze technieken geeft een beeld van de structuur op de nanometer schaal, de micrometer schaal en op de macroscopische schaal (rheometrie).

De structuur van vaseline is sterk van temperatuur afhankelijk. Wanneer vaseline verwarmd wordt, smelt het en gedraagt het zich meer als een olie. Wanneer het vervolgens afgekoeld wordt, begint het te stollen en weer de typische halfvaste structuur te vormen die bekend is van vaseline en zalven. De metingen laten zien dat vaseline bij kamertemperatuur voor 21% uit vast materiaal bestaat. Deze vaste fractie blijkt uit lamellen te bestaan. Deze lamellen ordenen zich in stapels. Dit soort stapels kunt u zich voorstellen als raamlamellen die naast elkaar ofwel onder elkaar geordend zijn. De lamellen in vaseline verdwijnen gedurende opwarmen en ontstaan weer wanneer de vaseline afgekoeld wordt. In hoofdstuk 2.2 is het smelten en ontstaan van de lamellen afhankelijk van de temperatuur gevolgd. Parallel hieraan zijn ook de rheologische eigenschappen onder vergelijkbare condities gevolgd. Hieruit blijkt een directe link te bestaan tussen de aanwezigheid van lamellen en de mate van rigiditeit van vaseline. Wanneer meer stapels lamellen aanwezig zijn, is de vaseline meer rigide en dus moeilijker smeerbaar is.

Door middel van wide-angle X-ray scattering (WAXS) is ook de kristalliniteit opnieuw bestudeerd (verwijzend naar hoofdstuk 2.1). Hieruit blijkt dat de eerdergenoemde lamellen gedeeltelijk uit kristallijne domeinen bestaan. Deze kristallijne domeinen zijn circa 50 nm groot en bestaan uit rhombohedrische eenheidscellen. Hieruit kan geconcludeerd worden dat de structuur van vaseline op de nanometer schaal bestaat uit kristallijne domeinen in de lamellen. Op de micrometer schaal bestaat vaseline uit stapels lamellen. De interactie tussen deze stapels lamellen zijn vervolgens verantwoordelijk voor de macroscopische eigenschappen van vaseline. Het is aannemelijk dat de stapels lamellen de vloeistof fractie van vaseline (olie) insluiten en zo verantwoordelijk zijn voor het halfvaste karakter. In hoofdstuk 2.1 is met name de rol van de kristallen als belangrijk aangemerkt. Hoofdstuk 2.2 laat zien dat niet de kristallen maar voornamelijk de lamellaire structuren de rheologische eigenschappen van vaseline sterk beïnvloeden. Daarbij is de X-ray diffractometer uit hoofdstuk 2.1 minder geavanceerd dan de synchrotron X-ray technieken uit hoofdstuk 2.2. Dit maakt de resultaten uit hoofdstuk 2.2 aannemelijker. Dit toont aan dat alleen door het gebruik van verschillende fysische karakterisatie methoden een volledig(er) beeld van vaseline verkregen kan worden.

Critical Process Parameters (CPPs)

In hoofdstuk 3 zijn de CPPs bestudeerd voor cetomacrogolzalf. De invloed van variabelen in het productieproces, zoals mengsnelheid en afkoelsnelheid op de smeerbaarheid van de zalf zijn op lab schaal (0,5 kg/batch) bestudeerd. Dit is gedaan door middel van

een zogenoemde Design of Experiments (DoE). Hiermee wordt een systematische reeks experimenten uitgevoerd om de invloed van de verschillende individuele variabelen op de smeerbaarheid te bestuderen. Productieprocessen zijn vaak complex. Het aanpassen van een variabele beïnvloedt vaak ook andere procesvariabelen. Denk bijvoorbeeld aan het aanpassen van de afkoelsnelheid. Hiermee kan ook direct de tijd van mengen, en daarmee de totale productietijd, ingekort worden. In een dergelijk geval is het lastig om de invloed van alleen de afkoelsnelheid goed in kaart te brengen. Juist door dit soort complexiteit is het gebruik van een DoE aan te bevelen. Hiermee wordt namelijk door middel van statistiek de invloed van individuele variabelen berekend. In het geval van hoofdstuk 3.1 is een nieuw type DoE gebruikt, namelijk een Definitive Screening Design (DSD). Dit type experimenteel design geeft meer informatie over de verschillende variabelen die bestudeerd worden en daarnaast is een beperkter aantal experimenten nodig.

Voor de productie van cetomacrogolzalf zijn de volgende variabelen bestudeerd: opwarmtemperatuur, afkoelsnelheid, mengsnelheid en de temperatuur waarbij de zalf uitgevuld wordt in tubes (de uitvultemperatuur). Ten eerste viel op dat slechts 5 van de 14 geproduceerde batches voldeden aan de voorafgestelde eisen aan de smeerbaarheid. Variaties in het productieproces hebben dus een belangrijke invloed op de smeerbaarheid van de zalf. Door middel van de DSD statistiek is gebleken dat de mengsnelheid en de uitvultemperatuur de twee belangrijkste variabelen zijn ($p = 0{,}0013$ en 0,0065 respectievelijk). Deze bevindingen zijn vervolgens vertaald naar industriële schaal. Deze staan beschreven in hoofdstuk 3.2.

Het opschalen van een productieproces is een belangrijke uitdaging binnen de farmaceutische industrie. Een manier om op te schalen is door eerst kennis van het productieproces op lab schaal te verkrijgen. Zodra men weet welke procesvariabelen kritisch zijn op lab schaal kan de nadruk gedurende het opschalen gelegd worden op deze kritische variabelen. Op basis van de lab schaal studie (hoofdstuk 3.1) is bij het opschalen daarom gefocust op de invloed van mengsnelheid en uitvultemperatuur op de smeerbaarheid van de zalf. Opvallend is dat de mengsnelheid, die zo kritisch bleek op lab schaal, geen belangrijke rol lijkt te spelen op industriële schaal. Het mengen van een product is zeer afhankelijk van het mengprincipe en de afmetingen van de productieapparatuur. Buiten het feit dat de lab en industriële schaal productie apparatuur totaal verschillend zijn wat betreft afmetingen en vorm, is ook gebruik gemaakt van een verschillend mengprincipe. Op industriële schaal is gebruik gemaakt

van zowel een menger als een homogenisator. Op lab schaal is alleen gebruik gemaakt van een menger. De homogenisator zal ongetwijfeld een belangrijke impact op het product hebben gehad. In een homogenisator wordt dusdanig veel kracht uitgeoefend op het product dat deze waarschijnlijk een sterke invloed zal hebben op de vorming van structuur in de zalf.

De uitvultemperatuur daarentegen blijkt wel kritisch op industriële schaal. Dit is vergelijkbaar met wat op lab schaal gevonden is. De invloed van uitvultemperatuur op de smeerbaarheid van cetomacrogolzalf lijkt daarom onafhankelijk van de schaal van productie of de gebruikte apparatuur. Het uitvulproces is echter uitdagender op industriële schaal dan op lab schaal. Op lab schaal kan men vaak volstaan met het simpelweg handmatig uitvullen van enkele tubes. Op industriële schaal gaat het om grotere volumina en is het belangrijk dat een bepaald aantal tubes per tijdseenheid accuraat gevuld worden. Dit stelt eisen aan de stromingseigenschappen van een product. Voor zalven zijn deze eigenschappen sterk temperatuur afhankelijk. Stromingseigenschappen van een product kunnen bepaald worden door het meten van een viscositeit, in dit geval een viscositeit afhankelijk van de temperatuur. Daarom is een boven- en ondergrens aan deze viscositeit vastgesteld waarbinnen een zalf accuraat en voldoende snel in tubes uitgevuld kan worden. Deze onder- en bovengrens worden ook wel “proces window” genoemd. Dit process window blijkt voor cetomacrogolzalf tussen 26 en 33 °C te liggen. Bij lagere en hogere temperaturen (hogere en lagere viscositeit respectievelijk) was het onmogelijk om de uitvulmachine juist te gebruiken. Zo spetterde het product op de verschillende machine onderdelen of werd het product niet meer homogeen over de tubes verdeeld. Dit proces window is vervolgens vertaald naar een aantal andere producten (waaronder zowel crèmes als zalven) en bleek universeel toepasbaar. Daaruit kan geconcludeerd worden dat het opschalen van een productieproces gebaseerd kan worden op proceskennis die is opgedaan op lab schaal.

Critical Quality Attributes (CQAs)

In hoofdstuk 4 zijn de CQAs bestudeerd voor triamcinolonacetonide (TCA) in een zalfformulering (TCA zalf FNA (Formularium Nederlandse Apothekers)). TCA is een corticosteroïde dat wordt toegepast bij ontstekingen op de huid bij ziekten als eczeem en psoriasis. In hoofdstuk 4.1 wordt de ontwikkeling en validatie van een stabiliteit indicerende analyse methode (HPLC-UV) voor de detectie van alle degradatie producten voor TCA in TCA zalf FNA beschreven. Deze methode is gevalideerd volgens de eisen die gesteld worden in de ICH (International Conference on Harmonisation) richtlijnen.

De degradatie van TCA is een oxidatief proces. In deze studie is een uitgebreid pakket aan oxidatieve stress testen gebruikt om de degradatie producten van TCA te kunnen voorspellen. Interessant genoeg bleek dat alleen door het toepassen van onconventionele tests als de toevoeging van azobisisobutyronitrile (AIBN) en zowel ijzer als koper zouten een compleet (representatief) beeld van de degradatie van TCA verkregen is. Dit is vervolgens geverifieerd door de resultaten te vergelijken met de degradatie in een TCA zalf FNA product dat 4½ jaar (1 ½ jaar langer dan de gestelde houdbaarheid) bij kamertemperatuur bewaard is gebleven. Deze analytische methode is vervolgens gebruikt om het mechanisme waarmee TCA degradeert in de zalf op te helderen.

In hoofdstuk 4.2 wordt de rol die hulpstoffen in de zalf spelen in de degradatie van TCA beschreven. Hulpstoffen als wolvet en vaseline blijken de degradatie van TCA significant te versnellen. Door middel van de techniek inductive coupled plasma optical emission spectrometry (ICP-OES) is aangetoond dat wolvet en vaseline zeer kleine hoeveelheid aan metalen zoals koper, ijzer en nikkel bevatten. In de zalf bleken deze sporen zich op te hopen in het propyleenglycol gedeelte van de zalf. Dit is het gedeelte waarin ook de TCA zich bevindt vanwege de hogere oplosbaarheid van TCA in propyleenglycol. Het is nu aangetoond dat de ophoping van deze metaalsporen in propyleenglycol verantwoordelijk is voor de versnelde degradatie van TCA.

Van metaalsporen is het bekend dat deze in staat zijn om oxidatieve reacties te katalyseren. Koperacetaat is gebruikt als model voor de metaalsporen in de zalf. De degradatieconstante van TCA bleek hoger in aanwezigheid van hogere concentraties koperacetaat. Daarnaast is het afbraak mechanisme (degradatie) van TCA opgehelderd. De alcohol groep in de 20-keto-21-hydroxyl zijketen van TCA oxideert eerst tot een aldehyde. Deze component degradeert vervolgens tot vier andere producten. Van de vorming van de aldehyde is aangetoond dat dit gekatalyseerd wordt door metalen. Deze 20-keto-21-hydroxyl zijketen is echter niet uniek voor TCA. Sterker nog, het grootste gedeelte van de andere farmaceutisch beschikbare corticosteroïden bevat deze zijketen. Daarom zijn de bevindingen uit hoofdstuk 4.2 vertaald naar andere corticosteroïden in hoofdstuk 4.3. Hiervoor zijn, naast TCA, hydrocortison (HC), desoximetason (DS) en betamethason-17-valeraat (B17V) bestudeerd. In eerste instantie begon dit als een review waarbij het doel was een overzicht te creëren van de beschikbare literatuur over de degradatie van dit soort corticosteroïden. Wat echter is gebleken is dat onvoldoende literatuur beschikbaar is over dit specifieke onderwerp, met name over de verschillen in degradatie in waterig en niet-waterig milieu. Dit is met name relevant voor de corticosteroïden die toegepast

worden in zowel zalven (niet-waterig) en crèmes (gedeeltelijk waterig). Daarom is eigen data verzameld om de hiaten in de literatuur in te vullen en zo tot een compleet beeld te komen. HC, DS en TCA hebben de eerdergenoemde 20-keto-21-hydroxyl groep gebonden aan het 17^{e} koolstofatoom in het steroïd molecuul. Naast deze 20-keto-21-hydroxyl groep zijn nog andere groepen aan dit 17^{e} koolstofatoom gebonden. Deze zijn voor HC, DS en TCA verschillend. Er is aangetoond is dat deze groep (die dus verschillend is voor de verschillende corticosteroïden) de manier van degraderen en de snelheid van degraderen sterk kan beïnvloeden. Voor de bestudeerde corticosteroïden blijken er zeven verschillende degradatie producten te kunnen ontstaan. HC is in staat om te degraderen tot het grootste aantal verschillende degradatie producten (namelijk vijf) en liet mede daardoor ook de snelste ontleding zien. Daarnaast is de transesterficatie van B17V bestudeerd in zowel waterig als niet-waterig milieu. Op basis van alle bovengenoemde resultaten is het complete reactiemechanisme beschreven voor corticosteroïden in waterig en niet-waterig milieu.

Samenvattend geeft dit proefschrift weer dat de kwaliteit van een zalf afhangt van eigenschappen van (1) de verschillende grondstoffen, (2) het productieproces en (3) de formulering. Door het toepassen van een systematische (QbD) benadering is de benodigde product en proces kennis verkregen. De verkregen kennis over vaseline, zalfproductie en corticosteroïd degradatie kan direct in de praktijk toegepast worden.
Zo weten we nu dat vaseline een farmaceutische grondstof is waar belangrijke verschillen in consistentie in bestaan. Nu weten we ook dat dit, naast verschillen in samenstelling, veroorzaakt wordt door verschillen in lamellaire structuuropbouw. Deze bevindingen kunnen toegepast worden in de (her)ontwikkeling van zalven en crèmes die vaseline of soortgelijke grondstoffen bevatten. Daarbij is ook duidelijk geworden dat bij het ophelderen van de structuur van dit soort materialen alleen volstaan kan worden door het toepassen van een breed scala aan verschillende technieken. Alleen op die manier kunnen alle puzzelstukken bij elkaar verzameld worden.

Het opschalen van productieprocessen zal hoe dan ook een uitdaging blijven in de toekomst. Echter onderschrijft dit proefschrift het belang van product en proces kennis op lab schaal alvorens op te schalen. Hierdoor kan namelijk de hoeveelheid experimenten op industriële schaal sterk verlaagd worden.

De chemische ontleding van corticosteroïden is nu een stuk duidelijker en inzichtelijker geworden. Deze belangrijke geneesmiddelgroep wordt veelvuldig toegepast in onder andere zalf en crèmeformuleringen. Door het ophelderen van de ontleding in vergelijkbare milieus kan in de toekomst tot kwalitatief betere producten gekomen worden. Hoewel tot in het recente verleden aangenomen werd dat bijvoorbeeld de TCA zalf FNA stabiel zou zijn, omdat immers de crème formulering stabiel is, blijkt nu dat juist voor dit soort ”oude” formuleringen vandaag belangrijke verbeterslagen te maken zijn. In die zin mag dit proefschrift gezien worden als een aanzet tot de herontwikkeling van ”oude” formuleringen. Een groot deel van de geneesmiddelen dat vandaag de dag door patiënten gebruikt wordt bestaat uit dit soort ”oude” formuleringen. Wanneer men bedenkt dat deze ontwikkeld zijn middels inmiddels verouderde methoden en eisen, lijkt het duidelijk dat hier grote winst te behalen is.

Appendix A.3

List of coauthors

List of coauthors

Affiliations at the time the research was conducted.
Presented in alphabetic order.

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Appendix A.4

List of publications

List of publications

Research articles:

- Elucidation of the variability in consistency of pharmacopoeia quality petrolatum. **van Heugten AJP**, Versluijs-Helder M, Vromans H.
  Drug Dev Ind Pharm. 2017;43(4):595-9.

- The influence of cetomacrogol ointment processing on structure: a definitive screening design.
  van Heugten AJP, Braal CL, Verluijs-Helder M, Vromans H.
  Eur J Pharm Sci. 2017;99:279-84.

- Development and validation of a stability-indicating HPLC-UV method for the determination of triamcinolone acetonide and its degradation products in an ointment formulation.
  van Heugten AJP, de Boer W, de Vries WS, Markesteijn CMA, Vromans H.
  J Pharm Biomed Anal. 2018; 149:265-70.

- The role of excipients in the stability of triamcinolone acetonide in ointments. **van Heugten AJP**, de Vries WS, Markesteijn CMA, Vromans H.
  AAPS PharmSciTech. 2018.

- Topically used corticosteroids: what is the big picture of drug product degradation? **van Heugten AJP**, de Boer W, de Vries WS, Pieters RJ, Vromans H.
  Eur J Pharm Sci. 2018;117:1-7.

- Study of petrolatum structure: explaining its variable rheological behavior. **van Heugten AJP***, Landman J*, Pethukhov AV, Vromans H. *both authors contributed equally
  Int J Pharm. 2018;540(1-2):178-84.

Publicaties in populaire “high-impact” journals

Mijn dank is groot aan een van mijn paranimfen met wiens hulp het startschot gegeven is voor deze dissertatie. Namelijk de officieus eerste publicatie in het populaire weekblad de Donald Duck. In een editie uit december 2016 is namelijk een “letter to the editor” gepubliceerd welke hieronder is bijgevoegd.

Donzige Duck. U bent vaak een pechvogel, maar ik kan er ook wat van! Ik ben al jaren bezig met mijn promotieonderzoek naar de werking van zalven en crèmes. Ik moet voor mijn promotie minstens drie publicaties hebben, maar ondanks het feit dat ik bij allerlei tijdschriften artikelen heb ingediend, is dat nog niet gelukt. Wilt u mij daarom in uw tijdschrift publiceren?

Anton van Heugten

Ik hoop dat dit plekje een zalvende werking heeft, Anton!

Appendix A.5

Dankwoord

Dankwoord

Dan nu het dankwoord van dit proefschrift. Ik heb dit tot het laatste moment uitgesteld en het is eigenlijk precies zoals ik het mij voorstelde. Een vrijdagmiddag in alle rust thuis met een heerlijke Westmalle Tripel, verlost van de wetenschappelijke stijlregels, wat welgemeende woorden op het digitale papier doen verschijnen.

Zoals u ongetwijfeld weet is het promotietraject voor mij een bijzondere periode geweest. Ik begon er aan omdat ik graag een sprong in het diepe wilde wagen en dat is gelukt! Hierbij denk ik echter ook aan de onverwachte gebeurtenis nu een jaar geleden, namelijk het geconfronteerd worden met een nare ziekte, teelbalkanker. Mede met dat in mijn achterhoofd wil ik een groot aantal van de mensen die ik in dit dankwoord zal noemen (en ook velen die ik hier niet specifiek noem (!)) extra bedanken. Het moge duidelijk zijn dat bij wat deze mensen voor mij het afgelopen jaar betekend hebben, de eventuele steun gedurende het promotietraject verbleekt. Niets dan respect en bewondering daarvoor!

Herman, onze eerste kennismaking vond plaats op station Assen, met een koffie bij de Smullers om precies te zijn. Ik had je gevraagd om eens wat te vertellen over het werken in de farmaceutische industrie en al snel bleek het tussen ons te klikken. Binnen het half uur vroeg je mij of ik geïnteresseerd zou zijn om onderzoek te gaan doen bij een grootschalige bereidingsapotheek. Wat mij betreft heb je hiermee het startschot gegeven voor een voor mij buitengewoon leerzame periode. Je bent een begeleider met een helder oog voor de sterke en zwakke punten van de promovendus. Hierdoor heb je mij veel over mijzelf geleerd en heb ik het idee vooral ook als persoon gegroeid te zijn. Jouw ”plons-methode” heeft mij middels vallen en opstaan veel gebracht. Op wetenschappelijk vlak heb je mij uiteraard ook veel geleerd. Ik ken geen persoon die zo scherp tot de basis van een vraagstelling kan komen als jij, daar heb ik bewondering voor! Daarnaast ben je als mens erg sociaal en hebben we vooral ook veel lol gehad. Hierbij denk ik aan onderzoekbesprekingen, hei dagen, congressen en (team)uitjes. Herman, dank voor je ondersteuning en vertrouwen!

Hans en Jos, als experiment kwam ik binnen bij Tiofarma en ik ben jullie in het bijzonder enorm dankbaar voor de kansen die jullie mij gegeven hebben. Als eerste werknemer binnen de R&D, heb ik de R&D afdeling ”vorm mogen geven”. Hierbij ben ik naar mijn idee vaak als ongeleid projectiel allerlei ideeën gaan uitvoeren. Met geduld, coaching en

vooral een gedeeld enthousiasme denk ik dat we tot iets moois gekomen zijn en, vooral met de hulp van vele nieuwe collega's, bouwen aan nog veel mooiers. Mede dankzij jullie voel ik mij onderdeel van het bedrijf en kan ik mijn creativiteit volledig kwijt. Daarnaast blijken jullie niet alleen in goede tijden maar ook in mindere tijden een belangrijke steun in de rug. Dank!

De beoordelingscommissie van dit proefschrift bestond uit Prof. Dr. J.A. Bouwstra, Prof. Dr. H.W. Frijlink, Prof. Dr. G. Van den Mooter, Prof. Dr. R.J. Pieters en Prof. Dr. A.C.G. Egberts. Hartelijk dank voor het beoordelen van mijn manuscript.

Collega's van de R&D-afdeling: Wouter, Dick, Herman, Sandra, Thijs, Marian, Merel, Marc, Feike, Yvonne, René, Arend, studenten en oud-collega's Eva en Myrthe, dank voor de samenwerking en afleiding. In het bijzonder wil ik Wouter en Marian bedanken voor de intensieve samenwerking om de degradatie van TCA op te helderen. Ik heb dit als buitengewoon plezier ervaren en vond vooral de afwisseling tussen grappen ("bèèèh, wolvet", "de schoorsteen", "Nee, ik ben niet-sociaal", etc.) en serieuze zaken (degradatieschema's tekenen en bediscussiëren) buitengewoon leuk en constructief!

Verder wil ik de andere collega's bij Tiofarma ook bedanken. Ik heb altijd een enorme betrokkenheid van de organisatie bij mijn onderzoek gevoeld en zijn er dan ook te veel mensen om hier op te noemen betrokken bij geweest. Aan allen dank!

Gedurende het promotieonderzoek heeft het begeleiden van bachelor en master studenten ook een belangrijke rol gespeeld. Wouter, Corné, Yuan, Tomy, Louwrens, Carlo en Wiebrand dank voor jullie inzet gedurende jullie masteronderzoek! Merijn, Sanne en Aisha dank voor jullie inzet gedurende jullie bacheloronderzoek!

Van de Pharmaceutics vakgroep in Utrecht wil ik in het bijzonder de docenten (Henk, Thom, Mario, Nel, Marie-Louise, Gerda, Christien, Frederique, Anne-Marie, Everaldo, Robbert Jan, Herre, Ivonne, Marie-Jose, Tamanna en Ellen), Raween (gedurende je promotietraject) en daarnaast Mies en Barbara bedanken voor de steun en afleiding gedurende deze periode!

Roland, Marjan, Andrei en Jasper dank voor jullie hulp bij het tot stand komen van een aantal hoofdstukken uit dit proefschrift!

CsO-wolfpack (Joepiooo, Loueh, Remt en Salantino), dank voor jullie steun afgelopen jaar en vooral voor de afleiding gedurende de rest van de periode!

Oma ook jou wil ik hier in het bijzonder noemen. Ik bewonder het feit dat je zo scherp en outgoing bent. Hierbij denk ik vooral aan het weekendje Londen, samen met mijn broer Stef, drie jaar geleden. Je reactie aan de telefoon “Oh jullie willen me meenemen voor een verrassingsweekend in het buitenland en ik mag niet weten waarheen? Dat is goed!” is en blijft legendarisch! Dank ook verder voor je steun en ik hoop dat we nog lang mogen filosoferen en lachen samen!

Rixt, het moge duidelijk zijn dat ik je dankbaar ben voor alles de afgelopen jaren. We vormen een goed team en maken vooral ook veel plezier. Laten we dat vooral blijven doen!

Dan nu tot slot mijn familie, Mam, Pap, Stef en Vera, (Jock en Perro),
Dank voor jullie onvoorwaardelijke steun, al ver voor dit promotietraject. Ik ben jullie enorm dankbaar voor alles wat jullie gedaan hebben en ben we er van bewust dat ik zonder deze steun niet had gestaan waar ik vandaag sta. Met name de term onvoorwaardelijk heb ik inmiddels leren kennen en dus prijs ik mijzelf gelukkig dat ik jullie heb!

Appendix A.6

Curriculum vitae

Curriculum vitae

Ton van Heugten was born on May 26th 1989 in Eindhoven. After graduating from pre-university education at the Carolus Boromeus College Helmond in 2007, he went to study Pharmacy at the University of Groningen. Here he received his Bachelor and Master of Science degree. He conducted his scientific internship at the department of Pharmaceutical Technology and Biopharmacy at the Rijksuniversiteit Groningen. In this research he developed a dual-release, double coated tablet formulation using the ColoPulse technology. In 2014 he started his PhD research at the department of Pharmaceutics of the Utrecht Institute of Pharmaceutical Sciences and the Research & Development department at Tiofarma B.V. supervised by prof.dr. H. Vromans. During this PhD research Ton was appointed a 0.3 FTE teacher position at the Pharmaceutics department of the University of Utrecht. The PhD research resulted in this dissertation, which he will defend at the University of Utrecht on June 20th 2018.