ABDULLAH ALI TOPICAL FORMULATIONS, DESIGN AND DRUG DELIVERY

"A Dive into Water"

MALMÖ
UNIVERSITY

**TOPICAL FORMULATIONS,
DESIGN AND DRUG DELIVERY**

"A Dive into Water"

Malmö University
Health and Society,
Doctoral Dissertation 2021:3

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Front Illustration: Coral emulsion droplets

ISBN 978-91-7877-203-2 (print)

ISBN 978-91-7877-204-9 (pdf)

ISSN 1653-5383

DOI 10.24834/isbn.9789178772049

Printing: Holmbergs, Malmö 2021

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Malmö University, 2021
Faculty of Health and Society

The publication is also available at:
Mau.diva-portal.org

To Zahraa

You have Power over your Mind – not the outside events.
Realize this, and you will find Strength.
- Marcus Aurelius

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ABBREVIATIONS

API	Active Pharmaceutical Ingredient
O/W	Oil in Water
W/O	Water in Oil
TEWL	Transepidermal water loss
a_w	Water activity
RH	Relative humidity
PEG1500	Polyethylene glycol 1500
PBS	Phosphate-buffer saline
ADME	Absorption, distribution, metabolism, and excretion
MCC	Mucociliary clearance
HLB	Hydrophilic-lipophilic balance
NMF	Natural moisturizing factor
SC	Stratum corneum
OSA	Octenyl succinic anhydride
DSC	Differential scanning calorimetry
NMR	Nuclear magnetic resonance
FTIR	Fourier Transfer Infrared Spectroscopy
SAXS	Small angle X-ray scattering
WAXS	Wide angle X-ray scattering
SEM	Scanning electron microscopy
WETSEM	WET Scanning electron microscopy
Cryo-SEM	Cry-scanning electron microscopy
E-SEM	Environmental scanning electron microscopy
AFM	Atomic Force Microscope
PLS	Partial least square

ABSTRACT

Water is a vital component regulating the properties of topical formulations and their interaction with biological barriers, such as skin and mucosa. Changing the water content within the frame of the pharmaceutical triangle will have a huge impact on which type of formulation, such as a cream, ointment, gel, or lotion, is formed, as well as the physical properties of the formulation. The composition of a formulation, and the subsequent reformulation after application, will govern the features of the residual film. This will in turn affect the barrier properties of the underlying tissue and consequently the penetration of various substances across skin or mucosa.

The primary aim of this thesis has been to provide further understanding on differences between traditional surfactant-based formulations and particle-stabilized, Pickering, formulations and how specific excipients, like alcohols, emollients, and thickeners can affect their physical and/or sensorial properties. The secondary aim has been to gain more knowledge on the role of water in topical formulations and how it affects the properties of the underlying tissue on application.

By combining a portfolio of physicochemical techniques combined with sensory science, we have been able to identify differences between Pickering and surfactant-stabilized formulations. Starch-based Pickering emulsions were perceived as less greasy and sticky than traditional creams, even at high oil content. Moreover, we were able to develop a novel type of alcohol-based Pickering emulsion with combined moisturizing and antiseptic properties. We have also been able to link sensory attributes, evaluated by human volunteers, with physicochemical characterizations. Furthermore, the *in vitro* ForceBoard™ method was developed further and we evaluated its potential to be used as an *ex vivo* method using excised skin. In addition, we have shown that the water gradient over a biological barrier has a general relevance with respect to drug absorption and should be considered not only in dermal drug delivery but also for buccal and nasal drug delivery.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Den grekiska filosofen Thales konstaterade redan ca 600 f.Kr. att vatten är grunden till allt som existerar (*arkhe*). Inte illa gissat, då vatten är det vanligaste ämnet på Jorden och förekommer i form av ånga, vätska eller is (fast form). Det krävs bara en tanke på de blå haven, glaciärerna, de svävande molnen och alla växter med sina vattenfyllda blad för att inse hur viktigt vatten är. Vatten är dessutom en viktig och livsavgörande beståndsdel i vår kropp. Den är viktig för funktionen av våra vävnader och organ, och för att upprätthålla en fungerade barriär mot främmande ämnen. Vatten är vanligt förekommande i hygien- och hudvårdsprodukter. Vatten är också en viktig byggsten vid utveckling av läkemedel såsom kortisonkrämer, smärtlindrande geler, nässpray och orala lösningar. Vatten styr dessutom hur läkemedelssubstanser kan transporteras genom huden och slemhinna.

Ett läkemedel eller en kosmetisk produkt som appliceras på en viss yta på kroppen brukar kallas för en topikal formulering. Det kan till exempel vara en kräm som appliceras på huden, en nässpray i kontakt med nässlemhinnan eller munspray som appliceras i kontakt med munslemhinnan. För att en topikal formulering ska klassas som ett läkemedel krävs det att det innehåller en läkemedelssubstans som har effekt på sjukdomar. En enkel topikal formulering kan i princip skapas av endast två eller tre ingredienser: vatten, olja och/eller pulver. Genom att variera sammansättningen av dessa tre ingredienser kan man påverka vilken typ av formulering som skapas, som till exempel kräm, salva, gel, suspension eller lösning. I den här avhandlingen har vi mest arbetat med formuleringar som till största delen består av vatten. Vilken typ av formulering som används kommer att påverka egenskaperna vid appliceringen, vid den efterföljande förändringen när flyktiga medel (vatten) avdunstar, och slutligen karaktären hos filmen som blir kvar på ytan där formuleringen har applicerats.

I den här avhandlingen har vi studerat två typer av topikala formuleringar, nämligen emulsionsbaserade hudkrämer och lösningar för nasal och buccal användning. En emulsion är en blandning, eller dispersion, av två icke blandbara vätskor såsom vatten

och olja. En emulsion skapas genom att sönderdela den ena vätskan, oljan i det här fallet, till små droppar som svävar omslutna av vatten. Förr eller senare kommer dock oljedropparna att slås samman så att oljan och vattnet separerar till två olika faser. För att stabilisera en emulsion och behålla oljedropparna intakta, brukar man tillsätta tensider. Tensider är små molekyler som har en lipofil (fettälskande) del som adsorberar till oljedropparna, och en hydrofil (vattenälskande) del som är i kontakt med vattenfasen. Vissa tensider kan vara irriterande för huden och ha negativ påverkan på miljön. Därför finns det ett stort intresse av att hitta nya ingredienser som kan ersätta tensider och skapa tensidfria emulsioner. Ett sätt att skapa tensidfria emulsioner är att använda partiklar från naturliga råvaror, så som stärkelse, för att stabilisera emulsionsdropparna i den omgivande lösningen. När man använder partiklar istället för tensider för att stabilisera emulsionsdroppar brukar man tala om Pickering-emulsioner. Vi har använt oss av stärkelse från Quinoa för att skapa Pickering-emulsioner.

De fysikalkemiska egenskaperna hos ett topikalt läkemedel kan påverka transporten av den aktiva läkemedelssubstansen genom hud och slemhinna. Vi har visat att om man till exempel har stora mängder av andra ämnen än vatten i sin formulering så kan bristen på vatten påverka barriäregenskaperna i vävnaden på ett sådant sätt att man får ett sämre upptag av läkemedelssubstansen. Det här är mycket viktigt att förstå och beakta så att man inte utvecklar läkemedel som motverkar sitt syfte, nämligen att på ett säkert och reproducerbart sätt leverera tillräcklig mängd av läkemedelssubstanser till kroppen. Extra allvarligt blir det om man vill ha ett snabbt upptag.

Det huvudsakliga målet med den här avhandlingen har varit att skapa en större förståelse för hur egenskaperna hos Pickering-emulsioner skiljer sig från traditionella tensid-baserade emulsioner, och hur olika typer av hjälpämnen påverkar bl a emulsionsstabilitet. Om emulsioner ska vara stabila över tid är det viktigt att motverka processer som leder till att oljedropparna blir större och att formuleringen så småningom därför separerar i en olje- och vattenfas. Som en del av den här avhandlingen har vi utvecklat stabila mjukgörande etanol-baserade Pickering-emulsioner. Dessa kan formuleras som ett mer hudvänligt alternativ till traditionell handsprit, men också som en bakteriedödande mjukgörande kräm. Fysikalisk stabilitet är dock inte det enda som är viktigt för en topikal formulering. Utseende, doft, och den upplevda känslan (perception) av en topikal formulering kan påverka huruvida produkten används på rätt sätt, och man vill därför kunna undersöka och styra faktorer som påverkar dessa egenskaper i formuleringsarbetet. Därför är det

viktigt att kunna korrelera de upplevda sensoriska attributen hos en formulering som man kan få fram med hjälp av en human panel till mer objektiva, och ofta billigare, karaktäriseringsmetoder. Här har vi vidareutvecklat metoder för att studera hur olika formuleringar påverkar friktionen mellan finger och kadaverhud. Vi har också använt andra fysikaliska mättekniker, såsom reologi (viskositet och flyt egenskaper) och hudhydrering (fukt i huden), för att studera formuleringars egenskaper och visat på hur dessa kan korreleras till upplevda sensoriska (estetiska) egenskaper som sammanställts med hjälp av en human panel. Sammantaget har vi lyckats förklara orsaker till upplevda skillnader mellan Pickering-krämer och traditionella tensidbaserade krämer. Vi har bl.a. kunnat konstatera att stärkelsebaserade Pickering-emulsioner upplevs som mindre kladdiga och klibbiga än traditionella krämer, även när oljehalten är hög.

Precis som matlagning, så är formuleringsarbete en blandning mellan konst och vetenskap men om vi ska kunna utveckla läkemedel snabbare och mer rationellt så måste vetenskapen vara den viktigaste ingrediensen. Precis som Vattnet. Den här avhandlingen har förhoppningsvis bidragit till detta.

ملخص علمي عام ARABIC SUMMARY

قال الفيلسوف اليوناني طاليس عام 600 قبل الميلاد أن الماء هو أساس كل الموجودات ، حيث أن الماء هو المادة الأكثر شيوعاً على الأرض ويوجد بشكل بخار، سائل أو جليد. لا يتطلب الأمر سوى التفكير في البحار الزرقاء والأنهار الجليدية والسحب و جميع النباتات التي تتكون من أوراق مملوءة بالماء لإدراك مدى أهمية المياه، وهو أيضاً عنصر مهم وحيوي في أجسامنا. إنه مهم لوظائف أنسجتنا وأعضائنا ، وللحفاظ على حاجز وظيفي ضد المواد المضرة. يوجد الماء بشكل شائع في منتجات النظافة والعناية بالبشرة. وهو أساس مهم في تطوير الأدوية مثل كريمات ضد الحساسية وبخاخات الأنف والمحاليل الفموية. كما يتحكم في كيفية انتقال المواد الفعالة في التركيبة عبر الجلد وأغشية الجسم.

في العادة يُطلق على مستحضر الدواء أو المستحضر التجميلي الذي يتم وضعه على سطح معين من الجسم اسم المستحضر الموضعي. ممكن أن يكون كريماً يوضع على الجلد ، أو بخاخ أنف يرش على الغشاء المخاطي للأنف أو رذاذ فم يوضع على بطانة الفم. عندما يكون المستحضر الموضعي منتج طبي فهو يحتوي على مادة طبية لها تأثير ضد الأمراض. من حيث المبدأ يمكن تشكيل تركيبة موضعية بسيطة من مكونين أو ثلاثة مكونات فقط: الماء والزيت و / أو المسحوق. تغيير تركيز هذه المكونات الثلاثة ، يؤثر على نوعية التركيبة التي تم إنتاجها ، مثل الكريم أو المرهم أو الجل أو المحلول. في رسالة الدكتوراه هذه استخدمنا الماء كأساس في تركيب المواد التي تم إنتاجها. ستأثر نوعية المستحضر المستخدم على مميزات التركيبة و طبيعتها عند الاستخدام، وفي الآخر على طبيعة الغطاء الذي يتكون على السطح الجسدي ، الجلد مثلاً، حيث تم استخدام التركيبة. درسنا في هذه الرسالة نوعين من التركيبات الموضعية ، وهما المستحلبات (مثل كريم البشرة) والمحاليل (للأنف وباطنة الفم).

المستحلب هو خليط من سائلين غير قابلين للامتزاج مثل الماء والزيت. يتم تكوين المستحلب عن طريق تحليل سائل واحد ، الزيت في هذه الحالة ، إلى قطرات عائمة محاطة بالماء. لكن بعد فترة ، تندمج قطرات الزيت بحيث ينفصل الزيت والماء إلى طبقتين مختلفتين. لإطالة إستقرار المستحلب والحفاظ على قطرات الزيت سليمة ، يتم عادةً إضافة المواد الخافضة للتوتر السطحي. وهي عبارة عن جزيئات صغيرة لها جزء محب للدهون يلتصق على قطرات الزيت ، وجزء محب للماء يتلامس مع الجزء المائي. بعض المواد الخافضة للتوتر السطحي يمكن ان ينتج عنها حساسية الجلد ولها تأثير سلبي على البيئة. لذلك ، هنالك اهتمام كبير بإيجاد مكونات جديدة لتحل محل المواد الخافضة للتوتر السطحي وانتاج مستحلبات بمكونات طبيعية.

تتمثل إحدى هذه الطرق الحديثه باستخدام جسيمات طبيعية ، مثل النشا ، لتثبيت قطرات الزيت في المستحلبات. يطلق على هذه النوعية من المستحلبات "بيكرينج" (Pickering) ، سميت بإسم العالم الذي اكتشف هذه الظاهرة اول مرة. لقد استخدمنا النشا من بذور الكينوا (Quinoa) لصنع مستحلبات البيكرينج.

يمكن أن تؤثر الخصائص الفيزيائية والكيميائية لمستحضر الدواء الموضعي على نقل المادة الفعالة عبر الجلد أو الأغشية الموضعية، مثلاً في الانف و الفم. لقد أظهرنا أن نقص الماء في المستحضر يمكن أن يؤثر على الغشاء بحيث تقل سرعة انتقال مادة الدواء عبر الغشاء .

لذلك ، من المهم جداً فهم هذا الأمر لتلافي تطوير تركيبات دوائية تتعارض مع هدف توصيل الأدوية بطريقة آمنة و سريعة. لقد استنتجنا هذا بشكل أساسي في التركيبات المستخدمة للأنف و بطانة الفم. كان الهدف الرئيسي من رسالة الدكتوراه هذه هو فهم و توضيح كيفية اختلاف خصائص مستحلبات بيكرينغ عن المستحلبات التقليدية القائمة على المواد الخافضة للتوتر السطحي. بالنسبة للمستحلبات ، من المهم أن تكون مستقرة ضد التغيرات مثل أن تصبح قطرات الزيت أكبر وأن التركيبة تنفصل في مرحلة الزيت والماء. وفي جزء من هذه الرسالة ، قمنا بتطوير مستحلبات بيكرينغ المعتمدة على الكحول والتي يمكن استخدامها كبديل للكحول اليدوي التقليدي للتعقيم حيث انها تتكون كلاً من الكحول القادر على التعقيم و الزيت المرطب للبشرة.

رغم اهمية تثبيت استقرار تركيبة المستحضر الطبي و اثبات امانها و فعاليتها، فهناك اشياء أخرى مهم ممكن أن تؤثر على مدى كيفية استخدام المريض للمستحضر بطريقة صحيحة و منتظمة، و هيا المظهر والرائحة والإحساس. لذلك يفضل أن يتم فحص هذه الخصائص والتحكم فيها من خلال التركيبة الكيميائية. لقد تمكنا من إثبات ان كريمات Pickering القائمة على النشا تعطي الاحساس بأقل لزوجة و شعور بالدهون من الكريمات التقليدية ، حتى مع استخدام الكثير من الزيت و كان ذلك مرتبطاً بوجود النشا.

كما عند الطهي ، فإن صياغة المستحضرات الموضعية عبارة عن مزيج من الفن والعلم ، ولكن إذا أردنا تطوير الأدوية بشكل أسرع وأكثر عقلانية، يجب أن يكون العلم هو العنصر الأكثر أهمية. تمامًا مثل الماء. نأمل أن تكون هذه الرسالة قد ساهمت في ذلك.

LIST OF PAPERS

This thesis is based on the following papers, which are cited in the text by their Roman numerals. The papers are appended in the end of the thesis.

- I. Relationship between sensorial and physical characteristics of topical creams: A comparative study of effects of excipients**
Abdullah Ali, Lisa Skedung, Stephen Burtleigh, Eva Lavant, Lovisa Ringstad, Chris D Anderson, Marie Wahlgren, Johan Engblom
Submitted to International Journal of Pharmaceutics
- II. Tactile friction of topical creams and emulsions: Friction measurements on excised skin and VitroSkin® using ForceBoards™**
Abdullah Ali, Lovisa Ringstad, Lisa Skedung, Peter Falkman, Marie Wahlgren, Johan Engblom
Submitted to International Journal of Pharmaceutics
- III. Alcohol-based Pickering emulsions as moisturizing topical hand sanitizers**
Abdullah Ali, Sara Thuresson, Chris D Anderson, Peter Falkman, Vitaly Kocherbitov, Lovisa Ringstad, Malin Sjö, Ana Skenderska, Marie Wahlgren, Johan Engblom
Manuscript
- IV. Will a water gradient in oral mucosa affect transbuccal drug absorption?**
Abdullah Ali, Marie Wahlgren, Lina Pedersen, Johan Engblom
Journal of Drug Delivery Science and Technology 48 (2018) 338–345
- V. Dehydration affects drug transport over nasal mucosa**
Abdullah Ali, Marie Wahlgren, Birgitta Rembratt-Svensson, Ameena Daftani, Peter Falkman, Per Wollmer, Johan Engblom
Drug Delivery, 26:1 (2019) 831-840

RELATED PUBLICATIONS

Related publications that are not included in this thesis.

- I. A Novel Technology for Personal Care Emulsions**
Abdullah Ali, Ecevit Yilmaz, Malin Sjöö
Sofwjournal, 141(2015) 2-7
- II. Use of non-dissolved starch based particles**
Malin Sjöö, Abdullah Ali, Jasmine Bedi
WO2019151936, filed February 2nd, 2018
- III. Sanitizing emollient**
Johan Engblom, Abdullah Ali, Malin Sjöö, Chris D Anderson, Marie Wahlgren
WO2020104553, filed November 18th, 2018

AUTHOR'S CONTRIBUTIONS

- I. AA did the main part of designing the study. Prepared the test formulations and performed all experiments, wrote documents for ethical approval with input from supervisors and managed the human sensory study. AA analyzed the results and wrote the manuscript with input from the co-authors.
- II. AA did the main part of designing the study and prepared the test formulations and performed most of the experiments. AA analyzed the results and wrote the manuscript with input from the co-authors.
- III. AA did the main part of designing the study and performed major parts of the experimental work and supervised a MSc-student (ST) who performed some of the experimental work and a BSc-student (AS) who contributed to the sensory study. AA designed the characterization experiments together with co-authors. AA analyzed the results and wrote the paper with input from co-authors.
- IV. AA performed parts of the experimental work and was the main author of the paper.
- V. AA did the main part of designing the study and performed major parts of the experimental work and supervised a MSc-student (AD) who performed some of the experiments. AA did most of the data evaluation of the experiments and made extensive contributions to writing the paper. AA submitted the paper as a corresponding author.

THESIS AT A GLANCE

PAPER I	Title Relationship between sensorial and physical characteristics of topical creams: A comparative study of effects of excipients	Aims Study possible relationships between physical properties and sensorial characteristics and understand how different excipients affect this, with particular emphasis on traditional surfactant-based creams and starch-stabilized Pickering creams.	Key findings Sensorial attributes related to the application and afterfeel phase of topical creams can be predicted by using rheological and tactile friction studies, respectively. Application of a Pickering cream resulted in high tactile friction which was associated with low afterfeel perception of residual coating stickiness, greasiness, and slipperiness.	Illustrations <p>The illustrations for Paper I include four graphs. Top left: A line graph of storage modulus (Pa) vs frequency (Hz) for various creams. Top right: A bar chart of relative change in pH (%) for different creams. Bottom left: A bar chart of normalized tactile friction coefficient for different creams. Bottom right: A bar chart of relative change in pH (%) for different creams.</p>
PAPER II	Tactile perception of topical creams and emulsions: Friction measurements on excised skin and VitroSkin® using ForceBoard™	Compare VitroSkin® and excised porcine skin as model substrates for tactile friction measurements of topical formulations. Detect possible differences between surfactant-based commercial creams and a starch-based Pickering cream.	When topical creams were applied, the same trends in results could be observed on VitroSkin® and excised skin, but there was difference in friction over time due to structural properties. Tactile friction of Pickering formulations is higher than for Canoderm indicating that Pickering formulations were perceived as less slippery.	<p>The illustrations for Paper II include six photographs. Top row: Two images of a person applying cream to their arm. Middle row: Two images of the ForceBoard™ device. Bottom row: Two images of the ForceBoard™ device.</p>

PAPER III	Alcohol-based Pickering emulsions as moisturizing topical sanitizers	Evaluate the potential use of Pickering emulsions to create a surfactant-free, user-friendly, and moisturizing alcohol-based hand rub	It was possible to create Pickering emulsions comprising a triglyceride, ethanol, and water with Quinoa starch particles as emulsion stabilizers. The stability of emulsions was confirmed after 3 years storage.
PAPER IV	Paper IV. Will a gradient in oral mucosa affect transbuccal drug absorption?	Evaluate the effect of dehydration on drug uptake through oral mucosa	An increase in water gradient may have a limiting effect on permeability through oral mucosa in similar way as for skin.
PAPER V	Paper V. Dehydration affects drug transport over nasal mucosa	Evaluate mucosal response to dehydration on nasal mucosa. How does water activity of formulations affect the nasal mucosa and its permeability?	An increase in water gradient over nasal mucosa induced substantial decrease in drug uptake for both Xylomethazoline HCl and ⁵¹ Cr-ETA. Similar results were obtained when comparing the two commercial products, Nasoferm® and Blox4®. Mucoadheson based on water sorption can slow down drug uptake in the nasal cavity.

1.INTRODUCTION

Topical formulations have become a big part of our everyday life. From the moment you wake up, you use soap and shampoo, moisturize your skin with a skin cream or body lotion, or disinfect your hands with your pocket hand disinfectant. Pharmaceutically it can be your decongestant nasal spray or mouth spray. There is a constant need to develop and improve topical formulations for different reasons. It could be because of reformulation of an expiring patent, a marketing strategy to use trendy ingredients or responding to rising ethical demands from consumers. It can also be because of need to solve a stability issue for an active ingredient or tuning the carrier to increase transport across topical membranes. A formulators task is to design the formulation matrix to live up to:

- Obtaining the right drug delivery profile.
- Functionality requirements, for example stability and appearance.
- Safety.
- Patient-compliance and cosmetic appeal.
- Costs and marketing.

The work in this thesis is divided into two parts, one dealing with how water affects formulation properties in topical drug delivery to nasal and buccal mucosa, and the other dealing with important aspects of formulation design such as the physicochemical- and sensorial properties of topical skin formulations.

There has been increasing interest in ways to modify topical formulations to overcome difficulties related to drug transport or to comply with consumer demands. This has often involved changing the ingredients or the composition of a formulation recipe. One of the main aspects is to change excipients such as surfactants towards more sustainable and innocuous ingredients. In this respect, we have worked with Pickering emulsions using starch particles from quinoa grains. Another key issue is how the formulation affects transport of active ingredients across topical membranes.

The primary aim of this thesis work has been to provide understanding on the difference between traditional surfactant-based formulations and Pickering formulations. This includes how formulation excipients can affect the physical and/or sensorial properties of topical products, and how these products interact with topical

membranes after application. The secondary aim has been to understand how water affects the topical membranes and topical formulations. Hence, we have looked at how water affects transport over topical membranes, skin hydration, and Pickering emulsion excipients. This includes, how to develop moisturizing ethanol-based hand rubs using Pickering emulsions.

More in detail, physicochemical and sensorial properties of particle-stabilized O/W creams were compared to conventional surfactant-based creams in paper I and II. The impact of the excipients on tactile perception was studied using a human sensory panel and by measuring skin hydration. In paper III, water was replaced by ethanol-water mixtures as the continuous phase of a Pickering O/W emulsion. The physicochemical properties of the resulting emulsions were studied, and tactile perception was evaluated by a human panel. In paper IV and V, we studied commercial formulations and aqueous solutions, comprising the same active pharmaceutical ingredient (API).

In this thesis, I will take the reader with me to a dive into water, and the sea of formulation science.

2. PERSPECTIVES ON WATER IN FORMULATIONS

Circa 600 BC, the Greek philosopher Thales claimed that water is the basic element (arkhe) of all existing things¹. Not a bad guess considering that water is the most abundant compound on earth, existing naturally in the forms of vapor, liquid, and solid. It is the main constituent of the cells, tissues, and organs in our body and is vital for life². In this thesis, water plays a big role related to topical formulations and their applications. This chapter will give a short overview of some of the scientific topics discussed in this thesis, focusing on the role of water. More detailed discussion on the scientific topics can be found in later chapters. But first, a dive into water...

Water – One corner in the pharmaceutical triangle

Because of its physiological compatibility and non-toxicity, water is the solvent most widely used as a vehicle in pharmaceutical products³. The unique properties of water as a solvent can be related to the polar oxygen-hydrogen bonds of the water molecule, resulting in formation of hydrogen bonds between water molecules and in similar favorable solvent-polar solvate interactions. Water is an important cornerstone of the pharmaceutical triangle for topical formulations, shown in Fig. 1. Without it, it would not be possible to create aqueous gels, suspensions, or emulsions. For instance, polymers in gels require hydration to swell and form a three-dimensional matrix throughout the hydrophilic liquid. Pharmaceutical colloids such as suspensions and emulsions comprise a dispersion of insoluble particles or liquid droplets in a continuous fluid phase. Emulsions usually consist of a mixture of two immiscible phases, an aqueous phase, and an oil phase. If the oil droplets are dispersed in a continuous aqueous phase, the emulsions are called oil-in-water (O/W). When water is dispersed in a continuous oil phase, the emulsions are called water-in-oil (W/O).

Figure 1. The pharmaceutical triangle illustrating the relationship in excipient composition of various topical formulations. Redrawn from Surber et al.⁴ with permission from S. Karger AG.

In this thesis, the topical formulations investigated fall within the lower right inner triangle in Fig. 1 where the intended use, drawing on patient and consumer need will determine the requirements and exact composition of the formulation⁴. The proportion of dispersed phase to aqueous continuous phase will affect the rheological properties of the formulation in the container and when applied on skin or mucosa. Evaporation of volatile solvents, like water or alcohol, from topical formulations will give a cooling and soothing sensation when applied to the skin⁵, and the subsequent reconstitution following evaporation will have certain tactile properties that will be important for patient compliance. Rapid evaporation of volatile compounds may also change the thermodynamic activity of the drug within the formulation, affecting the rate of drug transfer^{4,6}.

Hydration

Water content plays a crucial role in the homeostasis provided by the stratum corneum (SC), the outermost layer of our skin. The stratum corneum acts as the main barrier to percutaneous absorption of a wide variety of xenobiotics and prevents body desiccation in tough (dry) environmental conditions⁷. Hydration affects the physical properties of SC, such as permeability and flexibility, and is also thought to regulate enzymatic activities that are important for normal desquamation of the corneocytes at the skin surface⁸. At normal conditions, there is a water gradient between the viable

epidermis and the dryer ambient environment, creating passive diffusion of water from the inside towards the outside of the skin. This is referred to as transepidermal water loss (TEWL) and is regulated by the SC integrity. In dry skin of *e.g.*, atopic patients, TEWL is high indicating an impaired barrier function.

Moisturizers based on O/W emulsions are normally used as an attempt to slow down TEWL and promote restoration of the skin barrier function. Although these formulations are rich in water, the main part of the water quickly evaporates after application, leaving only a small amount of water to be absorbed by the SC. Instead, hydration of the skin is achieved by using occlusive excipients that prevent endogenous water loss from the skin, often in combination with humectants that attract and bind water. This is discussed in paper I and III.

Water also plays a role in topical formulations for oral and nasal drug delivery. Mucous membranes in the nose and mouth are normally well hydrated, whereas oral and nasal formulations often comprise polymer gels or powder-based formulations. Adhesion of such formulations is often driven by water sorption, inducing a higher water gradient over the tissue and subsequent dehydration⁹⁻¹⁵. This is discussed in detail in paper IV and V.

Water activity in formulations

Water activity (a_w) is defined as $a_w = p_w / p_w^0$, where p_w is the water vapor pressure of in equilibrium with the solution and p_w^0 is the water vapor pressure of pure water. It can also be explained as a value of how strongly the water is bound to the substance. Water activity ranges between 0 to 1, and the value of 1 represents pure water. The water activity can also be expressed in terms of the relative humidity (RH) of air as $a_w = p_w / p_w^0 = RH / 100$, and the chemical potential of water $\Delta\mu_w$ can be expressed in terms of a_w and RH as $\Delta\mu_w = RT \ln(a_w) = RT \ln(p_w / p_w^0) = RT \ln(RH / 100)$, where R is the gas constant (8.314 J/mol/K), and T is the absolute temperature.

In pharmaceutical applications, the water activity is mostly used as an indicator of water available for microbial growth. A strategy to minimize a product's susceptibility to spoilage is to reduce water activity by adding sugars and glycerol for oral products, or alcohols and glycols for topical products³. However, other common excipients used in topical formulations, such as polymers or humectants, can also lower the water activity of formulations^{11,16}. Furthermore, Björklund et al.^{11,17,18} has shown that a gradient in water activity across skin will affect drug permeability of drugs. Similar observations were made by Albér et al.¹⁹ who showed that common humectants, *i.e.*, urea and glycerol, promote skin hydration and drug permeability across the skin even at reduced water activity of the applied formulation. To understand if these

observations have a broader biological relevance, the effects on drug permeability of lowering the water activity of a formulation was also studied on buccal and esophagus mucosa (paper IV), and on nasal mucosa (paper V). Increasingly high amounts of polyethylene glycol 1500 (PEG1500) were used to lower the water activity of the formulations (up to 65 wt%, $a_w = 0.83$) and compared to formulations with phosphate-buffered saline (PBS) solution ($a_w = 0.996$).

The water activity in formulations can also affect the structural properties of some excipients. For instance, starch gelatinization occurs when starch is heated in the presence of water²⁰. It has been shown that using sugars^{21,22}, glycerol or ethanol²¹, to lower the amount of water available to the starch granules (*i.e.*, reducing the water activity), results in increased gelatinization temperatures.

In paper III the effect on starch gelatinization by replacing water with increasing amounts of ethanol was studied in quinoa starch dispersions. Such knowledge is vital for the preparation of alcohol-based Pickering emulsions, where it is important that the starch particles are in their granular form to adsorb to the oil droplets and stabilize the emulsion by forming a steric barrier.

Thales claimed that the earth floats on water, and similarly the scientific questions in this thesis floats on water.

3. TOPICAL DRUG DELIVERY

Drug delivery, *per se*, is the combined approach to administer a biologically active substance to the intended target site in sufficient amounts to achieve a desired pharmacological effect and minimize related adverse effects. This includes choice of delivery route and optimization of the preferred type of formulation.

To design a proper drug product we need to understand how the drug is absorbed, distributed, metabolized and eliminated in the body, a process often referred to as ADME^{3,23}. Pharmaceutical development demands that we understand how to influence these events by proper formulation design and making use of medical device technology. It is also important to bear in mind that a drug product normally contains a cocktail of various excipients, in addition to the drug, and that these also may be absorbed and affect the body.

Drug delivery is further often divided into local and systemic delivery. One benefit of local delivery is the potential reduction of adverse effects, as only the part of the body that needs treatment is exposed to the drug. Another benefit is the possibility to circumvent first-pass metabolism of the drug following oral systemic delivery, and a third aspect to consider is that the pharmacokinetics of oral drug delivery is relatively slow. However, systemic delivery is often needed to treat several target sites at the same time.

In this thesis the focus is on topical delivery and the role of water on drug transport. Topical delivery is primarily associated with delivery of various substances to or through the skin but relates to any product applied to a particular place on, or in the body. Hence, also buccal and nasal delivery falls well within the realm of topical delivery.

Local delivery

Topical formulations have traditionally been aimed for local delivery, either targeting the surface of the tissue or the skin or mucosa as such. In this thesis, the use of ethanol as a disinfectant could be considered as one example of a product targeting the surface, although rather classified as a biocidal than a cosmetic or pharmaceutical product. The use of moisturizing creams, comprising either occluding emollients or humectants retaining water, is considered as a local treatment for hydrating the skin interior. An ethanol-based Pickering emulsion could claim to be either a disinfectant or a moisturizing emollient or both. It could also easily accommodate a wide range of active ingredients and then be used for systemic drug delivery. The work on nasal and buccal delivery in this thesis are primarily focused on systemic delivery.

Systemic drug delivery

In systemic drug delivery, the main concerns are the transport of the drug across the biological barriers, and the contact time of the formulation at the delivery site.

Drug transport across biological membranes

Drug transport can either be active or passive. Active transport demands that the site of delivery carries live cells which have membrane-bound transport proteins. Passive transport, which is the main concern of this thesis, is based on diffusion of the active ingredient through the biological barrier. This is determined by Fick's law^{3,24}:

$$J = -\frac{D(x)}{RT} c(x) \frac{d\mu}{dx} \quad \text{Eq. 1}$$

Where $D(x)$ (cm^2h^{-1}) is the diffusion coefficient at position x (cm), $c(x)$ ($\mu\text{g cm}^{-3}$) is the concentration of the molecule at position x , and $d\mu$ (J mol^{-1}) is the chemical potential of the diffusing molecule. Fick's law gives us insights on the parameters that affect passive diffusion across membranes. The diffusion coefficient is both dependent on the properties of the diffusing molecule, and the permeability of the membrane. Thus, a solute molecule will have different diffusion coefficients in different biological barriers, as will be discussed later. The chemical potential (Eq. 2) of the drug is strongly related to the solubility of the drug in the drug vehicle. At concentrations far below the solubility limits, the chemical potential is unity and Fick's law is only dependent on the concentration gradient. However, as discussed in paper IV and V, for topical delivery it is beneficial for the uptake if the concentration of the API in the formulation is close to the solubility limit. Changes in the composition of the formulation can change the solubility of the API, and consequently

the driving force for transport across topical membranes. This is why considerable effort has been done, in paper IV and V, to determine the solubility of the active compounds. As an example, in paper IV, the solubility of Metronidazole in PBS was determined to be 1.17 wt% compared to 3.05 wt% in 65 wt% PEG1500 solution. The relation between chemical potential and drug activity can be realized from the following expression:

$$\mu_A = \mu_A^* + RT\ln(a_A) \quad \text{Eq. 2}$$

Where μ_A (J mol⁻¹) is the chemical potential of drug substance A, μ_A^* (J mol⁻¹) is the chemical potential of pure A, R (J K⁻¹mol⁻¹) is the gas constant, T (K) the temperature, and a_A the activity of A. Thus, in order to reach similar drug chemical potential in two different formulations, the drug activity needs to be considered at high concentration. While for very dilute solutions, the actual concentration can be used for comparison²⁵. In order to investigate other factors than the drug chemical potential, one has to take this into consideration. In paper IV and V, we did this by working at concentrations very close to the solubility limit of the different formulations or at concentrations far from the solubility limit where drug activity is not an issue.

Contact time at the active site

The contact time at the delivery site is both dependent on the formulation and the physical conditions at the site. For example, for nasal drug delivery the contact time is reduced due to mucociliary clearance (MCC). MCC limits the absorption by renewing the mucus layer lining of the mucus every 15-21 minutes²⁶⁻²⁸. In buccal delivery, the flow of saliva is constantly removing the dosage form from the delivery site²⁹. For skin, washing and contact with clothes etc. could affect the contact time. Thus, it is not surprising that considerable formulation effort has been put into increasing the contact time of the formulation with the biological membrane.

Strategies to increase the contact time, for nasal and buccal delivery, include the use of bioadhesive formulations. This could be sublingual buccal tablets that attach to the mucous membrane due to capillary forces^{29,30}, although it will probably lead to dehydration of the membrane^{12,30,31}. In other cases, both nasal and buccal formulations containing mucoadhesive polymers has been used^{32,33}. There are several theories that explain mucoadhesion, and one of them suggests dependence on hydration and swelling of the polymers. This indicates that this type of formulations could absorb water from the mucous membranes leading to dehydration of the membrane^{12,31,34}, which most likely could affect the transport of the drug across mucous membranes.

Effects of water on drug transport over topical membranes

It has been shown that hydration is important for drug permeation across skin^{11,35}. Previous studies in our group have shown that an increased water gradient can be detrimental to drug transport over the skin^{11,17–19}, as demonstrated by controlling the water activity of the formulations. Reduced water activity of the formulation will create a water gradient across the topical membrane that slows down drug transport. We wanted to investigate if the effects of the water activity gradient had a more general biological relevance for nasal and oral drug delivery. In paper IV, we studied the transport of three APIs; Metronidazole, Benzydamine, and Xylometazoline in solutions with different water activity across buccal and esophagus mucosa, and a silicone membrane. We used various amounts of PEG1500 to lower the water activity of the formulation. For example, the permeability of Metronidazole showed similar

Figure 2. A. Relative steady state flux of Metronidazole across mucosa (filled diamonds) and corresponding data on skin from Björklund et al.¹¹ (open diamonds) in formulations with different water activity. B. Steady state flux of Xylometazoline across mucosa from donor formulations comprising 2.0 and 2.7 wt% Xylometazoline in PBS (filled diamonds), and 2.0 and 4.5 wt% Xylometazoline in PEG1500 solution (crosses) respectively.

reduction in steady state flux across mucosa, as seen before for skin, see Fig. 2. However, the flux reduction was less pronounced for buccal mucosa compared to skin. Similar results were seen when Xylometazoline was used, where the flux was 40 times higher for PBS solutions than PEG1500 solutions (Fig. 2B). In paper V, a similar approach was extended to nasal mucosa, where we studied the potential effects of water activity gradients on nasal drug transport. The cumulative amounts of Xylometazoline permeated across nasal mucosa from two vehicles with different water activity and similar drug activity ($a_D = 0.68$) is shown in Fig. 3A. The steady-

state flux was circa 16 times higher for PBS solutions than PEG1500 solutions. Furthermore, two commercial vehicles, Nasoferm® and BLOX4®, were compared at the same drug concentration (0.1 wt% Xylometazoline), Fig. 3B, where Nasoferm® was found to be the better vehicle providing higher steady-state flux ($J_s = 29.5$ compared to $2.4 \mu\text{g cm}^{-2} \text{h}^{-1}$). Nasoferm® comprises a buffered solution, while BLOX4® was a continuous microemulsion based on glyceryl monooleate that could absorb water and is expected to dehydrate the mucosa³⁴. Thus, the difference in the results was connected to the gradient in water activity over the mucosa. This shows that the effects of water on drug transport, previously seen in skin, is of great importance for the transport of drugs over biological membranes.

Figure 3. A. Cumulative amount of Xylometazoline permeated across nasal mucosa from PBS (black diamonds) and 65wt% PEG (crosses) as vehicles, where the drug activity was set to ~ 0.68 ($n = 6$). B. Xylometazoline permeability through nasal mucosa from two commercial vehicles, Nasoferm® (circles, $n = 6$), and BLOX4® (triangles, $n = 3$).

4. TOPICAL FORMULATIONS

“Pharmaceutical formulation, is the process in which different chemical substances, including the active drug, are combined to produce a final medicinal product”

FDA

Skin creams, ointments, lotions, gels, or transdermal patches are examples of common formulation forms for topical delivery^{36,37}, as are nasal sprays and buccal patches. In this thesis we focus on two types of formulations, solutions, and emulsions.

Solutions

Aqueous solutions are used in a range of topical formulations such as nasal sprays and topical solutions³. While the dominating component in a solution is the solvent used, *e.g.*, water (paper IV and V) or ethanol (Paper III). Solutions might include several other excipients to give them the functional properties needed, such as polymers to affect viscosity, preservatives to increase microbiological safety, salt and buffers to control osmolality and pH, and surfactants to affect wetting.

Emulsions

Emulsion-based topical formulations exist in many forms in pharmaceutical and cosmetic products. The most common ones are creams and lotions. Of the two types of emulsions that can be produced (O/W or W/O), O/W emulsions are the most common as they have good cosmetic properties and can be produced at low cost with water as the continuous phase. In this thesis we are especially focusing one type of emulsions called Pickering emulsions (see chapter 5) and in our case these are stabilized by quinoa starch particles. Emulsions are thermodynamically unstable, and phase separation will eventually occur because of large interfacial tension between oil and water phases. The reason for this large interfacial tension between the two liquids is large difference in cohesion forces between them. The larger the difference in cohesion forces, the larger the interfacial tension³⁸.

Stability of emulsions

Physical stability

Instability of emulsions is mainly caused by four different mechanisms: creaming/sedimentation, flocculation, coalescence, and Ostwald ripening. The instability may be caused by a single process, or by different processes occurring simultaneously. Creaming/sedimentation occurs due to density differences between the two phases. Creaming is very common in O/W emulsions, where the oil phase has lower density than the water phase and the separation goes upward. The relationship between stability and the rate of gravitational separation that occurs in creaming/sedimentation can be elucidated by using Stokes' law, Eq. 3, and comparing it to the Brownian diffusion velocity, Eq. 4 and Eq. 5³⁹.

$$v_{Stokes} = \frac{g \cdot d^2 \cdot \Delta\rho}{18 \cdot \eta} \quad \text{Eq. 3}$$

where:

v_{Stokes} is the settling or creaming velocity (m s^{-1})

g is the gravitational acceleration (m s^{-2})

d is the droplet diameter (m)

$\Delta\rho$ is the density difference between dispersed and continuous phases (kg m^{-3})

η is the viscosity (Pa s)

$$|v_{diffusion}| = \sqrt{\frac{D}{2 \cdot t}} \quad \text{Eq. 4}$$

$$D = \frac{k_B \cdot T}{3 \cdot \pi \cdot \eta \cdot d} \quad \text{Eq. 5}$$

where:

$v_{diffusion}$ is the average Brownian velocity (m s^{-1})

D is the diffusion constant ($\text{m}^2 \text{s}^{-1}$)

t is the time (s)

k_B is the Boltzmann's constant (J K^{-1})

T is the absolute temperature in Kelvin (K)

In a short timescale the Brownian velocity will dominate over the Stokian velocity, and creaming/sedimentation will not occur. When the droplets are considerably larger the Stokian velocity will dominate over Brownian motions over longer timescales, and creaming/sedimentation will take place. If the droplet size is reduced the

Brownian velocity will increase, and it would take much longer time for creaming/sedimentation to occur. Other parameters that might influence the rate of gravitational separation are concentration, viscosity, and aggregation of droplets³⁹. Flocculation refers to the aggregation of droplets, where an energetically stable situation is reached, and droplets collide and attach to each other but still retain their integrity. In some cases, flocculation may be desirable to contribute to the formulation's rheology, but increased flocculation can lead to creaming and eventually lead to coalescence³⁸⁻⁴⁰. Coalescence is the process in which droplets fuse together to form new larger droplets, and eventually lead to complete phase separation. It can happen because of collisions or long time contact in the flocculate state or in a tightly packed cream layer. Creaming, sedimentation, and flocculation are reversible phenomena, meaning that a uniform dispersion can be regained by gently shaking the emulsion. However, coalescence can be seen as an irreversible phenomenon^{38,39}.

Another instability mechanism can occur due to differences in the internal pressure between smaller and larger droplets, creating a driving force towards larger droplets. This is referred to as Ostwald ripening and the excess pressure present is called Laplace pressure and is given by:

$$\Delta p = \frac{2\gamma}{r} \quad \text{Eq. 6}$$

where Δp is the Laplace pressure, γ is the interfacial tension between oil and water and r is the radius of the droplet³. Low solubility of the dispersed phase in the continuous phase slows down or prevents Ostwald ripening.

Chemical stability

The chemical stability of emulsions can be influenced by several effects. Examples include the compatibility of different excipients, such as the active ingredient or preservatives. Oxidation of oils is one of the main issues, especially for oils with a high degree of unsaturation. A way to solve this can be to incorporate antioxidants in the emulsion, such as vitamin E, and assure high ingredient purity⁴⁰.

Microbial stability

Contamination by microorganism can affect the physicochemical properties of emulsions. It is especially of importance for O/W emulsions where the water activity is high. The choice of ingredients, processing during production, and packaging will largely affect the microbial stability of the emulsions. One or more preservatives are normally added to prevent microbial growth. Care should be taken when choosing

preservatives, to understand how the pH and salt of the emulsion, as well as other excipients affect the activity of preservatives⁴⁰ and how the nature of the preservatives could affect the functionality of other excipients.

Excipients for topical formulations

Pharmaceutical excipients are substances other than the API that are intentionally included in an approved drug delivery system or a finished medicinal product.

Recalling the pharmaceutical triangle in Fig. 1, a simple topical formulation vehicle can be composed by combining two (or three) of the three cornerstones: water/solvent, oil, and powder. Other functional excipients are normally incorporated depending on the intended use of the product. Emulsifiers are needed to stabilize O/W emulsions, and thickeners may be supplemented to increase viscosity and add body to the formulation, and also slowing down the process of creaming/sedimentation. Humectants are hygroscopic compounds that function by retaining water and slowing down TEWL. Other excipients include preservatives to counteract microbial growth, salts as buffering agents, fragrances to mask odor from certain excipients, and skin feel agents to improve sensorial properties.

In this thesis, the role of emulsifiers (surfactants/Pickering), emollients, humectants (hydration), thickeners, and volatile compound (water/ethanol) have been studied with particular emphasis on their effect on the physical properties of the formulations in relation to tactile friction and sensorial properties.

Solvents

Water is normally used as a diluent and solvent and plays a crucial role in the hydration of other excipients, and hydration of topical membranes for drug delivery. Ethanol is another solvent relevant for this thesis (paper III). Alcohols in general, are normally incorporated as a carrier, solvent, penetration enhancer⁴¹⁻⁴⁴ or used as an antimicrobial preservative^{45,46}. Ethanol is the primary active ingredient in alcohol-based hand rubs, often in combination with isopropanol. Ethanol concentrations of 60 to 95 vol% are deemed safe and effective for disinfection, while the optimum concentration is generally considered to be 70 vol%⁴⁶.

Emollients

Emollients are usually oils (*i.e.*, triglycerides or mineral oil) or silicones (*i.e.*, dimethicone) that moisturize, soften, and lubricate the skin, which is usually based on occlusive effects that contribute to reducing TEWL. The chemical and molecular structure of emollients vary which affect their melting point, polarity, and

viscosity. The type of emollient used has been shown to affect skin feel, skin friction, and spreadability of topical formulations⁴⁷⁻⁵¹. In a topical emulsion, the dispersed phase may carry 5-30 wt% of emollients depending on if it is a lotion or a cream. Choosing the composition of emollients based on their viscosity and polarity, and combining this solid emollients allows the freedom to design the properties of the formulation based on the intended use⁵². Furthermore, emollient polarity will determine the solubility of an active ingredient and is an important aspect to consider when formulating for dermal drug delivery^{52,53}.

Surfactants

To stabilize emulsions, adsorption of an emulsifier at the oil-water interface is needed. Until now, the most commonly used emulsifiers are low molecular weight surfactants, which are amphiphilic molecules with a hydrophilic part, and a hydrophobic part that adsorb into the interface between oil and water and lower the interfacial tension. The hydrophilic part is normally called the polar headgroup, and the lipophilic part is referred to as the non-polar tailgroup^{38,54,55}. A rule of thumb in emulsion technology is the concept of hydrophilic-lipophilic balance (HLB) of a surfactant. The HLB number is expressed as a number based on the molecular weight of hydrophobic components compared to the molecular weight of the molecule^{38,40}. A downside of synthetic surfactants is that they could be toxic and/or cause irritation in patients with sensitive skin⁵⁶⁻⁶¹. Besides, their extensive use in other applications have also been cause for environmental concerns^{62,63}. In the search for alternative emulsifiers, there has been reports on using other ingredients such as proteins, polysaccharides and particles^{40,61,64,65}.

Humectants

Natural moisturizing factor (NMF) components are a mixture of low molecular weight, water-soluble compounds present in the SC with the ability to bind water when the skin is exposed to extreme dry conditions^{66,67}. Urea is an endogenous NMF that has been shown to reduce TEWL in atopic and ichthyotic patients⁶⁶. Urea is used in concentrations of 5% for the treatment of skin dryness⁶⁸ and higher concentrations for more severe skin disorders⁶⁹. Humectants are hydrophilic compounds that are capable to retain water within the skin by improving the water binding capacity of the SC to maintain hydration of the skin and minimize TEWL^{48,69,70}. They are often used in skin formulations to promote hydration. Humectants that are widely used include, among others, glycerin (also called glycerol), propylene glycol, and urea.

Glycerol is known to diffuse into the SC and retain water in the skin by forming a water-glycerol mixture that hydrates and plasticizes the skin, thus preventing dehydration in dry ambient conditions^{71,72}. The concentration of glycerol in creams can be as high as 20 %. In alcohol-based hand rubs glycerol concentrations range from 0.50 to 1.45 vol% as it was shown that it can decrease the bactericidal activity of the products^{73,74}. Propylene glycol is a frequently used alcohol with humectant properties. It is also used as solvent and penetration enhancer^{69,75}.

The effect of humectants on skin hydration was studied in skin creams in paper I *in vivo* (shown in Fig. 25) and *ex vivo*. The skin hydration was measured by means of electrical conductance (*in vivo*) and electrical capacitance (*ex vivo*) before and after application of skin creams. Humectants, in combination with occlusive emollients proved to be important for improved skin hydration. In comparison, a skin cream containing emollients in the absence of humectants did not improve skin hydration.

Viscosity modifiers

Viscosity modifiers or thickeners are added to a formulation to control its flow, giving it desired properties in terms of thickness, appearance, feel, and stability. Stabilization is an added value since increased viscosity will slow down creaming/sedimentation. Viscosity modifiers can be solids that require melting and incorporation to the dispersed oil phase, in-organic thickeners (*e.g.*, clays and silica), or polymer-based thickeners that hydrate and swell when dispersed in the continuous water phase. Solid thickeners include fatty alcohols (*e.g.*, cetyl alcohol), aliphatic alcohols (*e.g.*, isostearyl alcohol), fatty acids (*e.g.*, stearic acid), and natural and synthetic waxes that are normally present in topical skin products⁷⁶. Polymer-based thickeners can be natural or synthetic polymers and are widely used in pharmaceutical systems. Their properties depend on their molecular weight, the way the monomers are assembled (linear, branched, or crosslinked), and the monomer polarity⁷⁷. The most commonly used synthetic polymers, which are of particular interest in this thesis, are carbomers. Carbomer, also known as carboxypolymethylene or Carbopol, is widely used in pharmaceutical and cosmetic industries^{48,74,77,78}. It is a high-molecular-weight polymer of acrylic acid, with a high proportion of carboxylic acid residues. Carbomers are effective thickeners, suspending agents and stabilizers. When dispersed in an aqueous solution, carbomers are acidic and need to be neutralized with a base to reach the maximum viscosity between pH 6 and 11^{3,77}. Carbomers are used in paper I and II to add viscosity to a Pickering-stabilized, and surfactant-stabilized cream. The rheological properties were studied in paper I and

compared to surfactant-based creams with and without carbomers. The results show that creams with a carbomer displayed higher yield stress and viscosity, but the differences in rheological properties were also related to other excipients used. Large polymers, such as carbomers also have mucoadhesive properties, suitable for applications in oral and nasal drug delivery⁷⁷, and could in combination with high viscosity increase the contact time of the formulation with mucosa. However, this could also have drawbacks when it comes to drug delivery as discussed in chapter 3.

A closer look at two main excipients in this thesis

Two excipients that we have used in this thesis are triglycerides (triolein and tricaprin) and starch, which were studied deeper with X-ray diffraction.

Triglycerides

Triglycerides are oils that have been widely used in food^{40,79,80} and pharmaceutical formulations^{81–83}. The chemical structure of triglycerides is composed of three fatty acid moieties attached to glycerol. If all three fatty acids are the same, the triglyceride is termed a simple triglyceride. The chain length and degree of saturation of the

Figure 4. Complete picture of the polymorphic transitions of triglycerides together with schematic drawing of the chain packing sub cells. Redrawn from Hernqvist 1990⁸⁵. All transitions are irreversible except $\alpha \rightarrow \text{sub-}\alpha$ transition. The dotted lines indicate the possibility of direct crystallization in other forms than the α -form.

lipophilic part determines their properties⁸⁴. The longer the chains are, the higher will the melting point of the triglyceride be⁴⁰. Triglycerides are normally classified depending on the chain length of the fatty acid part into high (> 12), medium- (6-12), and low chain (< 6) triglycerides. The mostly used triglyceride in skin care products is a medium chain triglyceride, also known as MCT-oil, which is a mixture of caprylic/capric triglyceride (dry skin moisturizer).

The crystallization behavior of triglycerides may affect emulsion stability, and the key factors that determine if a triglyceride will crystallize are the temperature (melting point) and the type of polymorph that may form during crystallization. Triglyceride phases transitions between different polymorph forms (α , β' and β), where the β -form is the most stable one with the highest melting point. The polymorph crystalline structure corresponds to hexagonal-, orthorhombic-, and triclinic subunit cells respectively (Fig. 4). The α -form is the least stable form, with the lowest melting point. The β' -form exist in a metastable state, between α and β , with higher melting point than α but less than β . The liquid crystallizes into the α -form when cooled, and when it is cooled rapidly to very low temperatures it transforms to a sub- α form. When the α -form is stored just below its melting point, it is transformed first to the metastable β' -form, and finally into the stable β -form⁸⁵. This transition is the complete pathway for triglycerides to find the optimal packing of the molecules. The two β' -forms are quite similar, however they seem to differ in degree of crystallinity⁸⁶. Common techniques to study the thermal behavior and polymorphism of triglyceride include X-ray diffraction, nuclear magnetic resonance (NMR), and differential scanning calorimetry (DSC).

In this thesis we used triglycerides in paper II and III, with different chain length and degree of saturation, *i.e.*, tricaprinn and triolein, see Fig. 5. Tricaprinn is a saturated triglyceride of capric acid with three C_{10} chains, and it is solid at room temperature with a melting point for the β -form of $31.5\text{ }^{\circ}\text{C}$ ⁸⁷. Triolein is the unsaturated

Figure 5. Chemical structure of A. tricaprinn (C_{10}) and B. triolein ($C_{18:1}$).

triglyceride of oleic acid with three C₁₈ chains, with a *cis* double bond between carbon atoms nine and ten. It is liquid at room temperature with a melting point for the β -form of 4 °C⁸⁸.

In paper III, a simple ternary phase diagram was constructed for the triglyceride-ethanol-water systems to find the compositions where the dispersed and continuous phase of an emulsion are immiscible. The thermal behavior of triglycerides was studied with DSC, where tricaprinn was present in its β -form at room temperature (23 °C) while triolein was in liquid form. The triglycerides were further used to produce Pickering emulsions with various compositions of dispersed phase and ethanol-water mixtures.

The crystallinity of the triglycerides was studied with X-ray diffraction which confirmed that the crystalline structure of tricaprinn was not affected by the emulsion system. Furthermore, we observed a tendency for faster crystallization of tricaprinn in the alcohol-based emulsions than in the water-based emulsions, see Fig. 6. This was related to the lower melting point of tricaprinn in ternary tricaprinn-ethanol-water mixtures, possibly because of small amounts of ethanol being present affecting the kinetics.

Figure 6. WAXD data of water-based (PE TC:H₂O) and alcohol-based (PE TC:70%EtOH) tricaprinn Pickering emulsions with dispersed phase:continuous phase ratio of 3:7. A: PE TC:H₂O after 3 days storage (purple line) and after 1 month storage (blue line) at 25 °C. B: PE TC:H₂O after 3 days storage (orange line) and after 1 month storage (pink line) and after 3 years (dark blue line) at 25 °C. Tricaprinn in PE TC:H₂O was not yet crystalline 3 days after preparation, while in PE TC:70%EtOH it had already crystallized.

Starch

Starch can be found in many different plant parts, including fruits, seeds, tubers, and roots. Starch serves as an important energy source in the human diet in the form of cereal grains, and tubers^{89–91}. In addition, both native and modified starch is

considered as non-toxic and non-irritant materials and have been widely used as excipients in pharmaceutical formulations^{46,89,92}. Starch is a versatile excipient that has been readily used in pharmaceutical application in oral solid-dosage formulations as a binder, diluent and disintegrant. Fine starch powders, primarily from rice, wheat and corn starch, have been used in topical formulations as absorbents of liquids and also taking advantage of sensorial benefits^{46,76}. Starch has also been investigated as an excipient in nasal drug delivery⁹³. Modified starches have also been used as thickening agents and particle-stabilizers of O/W emulsions^{46,90,94–96}. Furthermore, starch granules are inexpensive, biodegradable, biocompatible, and can be isolated from many botanical sources. Particle size, shape, and composition of starch granules varies depending on the botanical source^{91,97}.

Quinoa starch

Quinoa (*Chenopodium quinoa* Wild) is a pseudocereal from the Andes in South America and dates back more than 5000 years. It was an important food for the Incas, who called it “the mother grain” and considered it as sacred. Recent interest in quinoa is related to its high-nutritional value with high content of quality protein, dietary fiber, lipids, and saponins^{98,99}. Furthermore, quinoa starch has a polygonal shape and small particle size (0.5–3 μm in diameter) with a unimodal size distribution^{90,99–101}, see Fig. 7. The amylose content of quinoa is relatively low in comparison to cereal starches, with reported values varying between 3.5–27 %^{99,100,102,103} with gelatinization peak temperatures (T_p) ranging from 50–70 $^{\circ}\text{C}$ ^{97,99–103}. However, the starch concentration, and consequently water content, varied in different studies which made comparisons difficult. Furthermore, different quinoa varieties^{99,104} and modifications^{105,106} can affect the physicochemical properties of quinoa starch.

Figure 7. Scanning electron microscopy image of quinoa starch particles and corresponding particle size distribution.

Modified starch as emulsifiers

In its native form, starch is not hydrophobic and is generally not suitable to adsorb at the oil-water interface. However, there are reports of successful use of native starch to stabilize emulsions^{96,97,107}, which was connected to the presence of surface bound protein in the starch granule^{96,97}. Nonetheless, the emulsifying capacity of native starch was low with large droplets. The hydrophobicity can be increased with either chemical^{96,97,108–114} or physical modification^{20,96,106,115,116} of the starch granules. Chemical modification of starch involve the reaction of starch with chemically substituted groups such as acetate or octenyl succinic anhydride (OSA)^{108,109}. The OSA-modified starches are obtained through an esterification reaction of starch hydroxyl groups and octenyl succinic anhydride under mild alkaline conditions (Fig. 8). The slightly basic conditions are thought to help reduce hydrogen bonding between starch chains in favor of swelling of the starch grains and diffusion of OSA molecules within the swollen granules. Due to the heterogenous conditions of the reaction, the substitution can be unevenly distributed between granules and the octenyl succinic groups are preferentially connected to at the surface of the granules¹⁰⁸. Several parameters have been identified for optimization of the reaction, such as starch concentration, level of OSA, mixing conditions, reaction time, temperature and solubility of OSA in the aqueous suspension^{110,112–114,117,118}. OSA-modified granular starches have been successfully used to prepare stable Pickering emulsions. Modified quinoa starch showed better emulsifying capacity compared to other starches^{96,97}, and the increasing the amount of OSA derived groups resulted in smaller emulsion droplets and higher aggregation of droplets^{97,119}.

Figure 8. Esterification reaction of starch granules with octenyl succinic anhydride. Adapted from Sweedman et al.¹⁰⁸ ©2013 with permission from Elsevier.

Structure of starch

Starch consists of two polysaccharides based on α -(D)-glucose, *i.e.* amylose, and amylopectin. Both consist of chains of α -(1,4)-linked D-glucose units interconnected with α -(1,6)-glycosidic linkages, forming branches in the polymers. Although

amylose was historically defined as a linear molecule¹²⁰, it is today well established that some amylose molecules contain to some extent α -(1,6)-linkages^{121–124}. Amylopectin is highly branched with short chains in comparison to amylose, and is composed of about 5% branched α -(1,6)-linkage bonds. The outer chains of amylopectin form double-helices that crystallize and contribute to the semi-crystalline nature of starch¹²¹. The composition of amylose/amylopectin varies among different botanical sources. For most starches, amylopectin constitutes the major part of starch granules (~70%), whereas amylose constitutes 15-30%^{121,125,126}.

Figure 9. Representation of the lamellar structure of a starch granule. Adapted from Qiao et al.¹³⁴©2017 with permission from Elsevier.

Starch granules are semi-crystalline, with alternating crystalline and amorphous parts. The amylopectin double-helices can crystallize into an A- or B-type, or a mixture of these two polymorphs, *i.e.*, the C-type^{121,127–130}. The properties of C-type starch depend on the distribution of A- and B-type polymorphs. The double-helices in the A-type crystal are closely packed into a monoclinic unit cell (with characteristic Bragg peaks at 5.8, 5.2 and 3.8 Å) containing 8 water molecules. In the B-type crystal, the double-helices are packed in a hexagonal unit cell (with characteristic Bragg peaks at 15.8-16.0, 5.9 and 5.2 Å) and can contain 36 water molecules. The C-type crystal is similar to A except for the appearance of a medium strong peak at about 16.0 Å. Illustrations of A- and B-type polymorphs are shown in Fig. 9. The presence of the 16 Å peak is related to the distance between amylopectin side chains and the lamellar structure (“the 100 inter-helix distance”), and is thought to depend on presence of moisture^{131–134}. The stacks of repeating crystalline and amorphous lamellae (Fig. 10) can be studied with small-angle X-ray scattering (SAXS) showing a 9-10 nm repeat distance in all starch granules^{121,132,135,136}. These stacks form ~100-400 nm shells or

rings. The rings are semi-crystalline since they contain both the amorphous and the crystalline lamellae and are normally thinner at the periphery, with thicker interior parts^{121,122}. In paper III, we have shown that quinoa starch, when hydrated, displayed the C-type polymorph.

Figure 10. Representation of the lamellar structure of a starch granule. Adapted from Tester et al.¹²² ©2013 with permission from Elsevier.

Gelatinization of starch

Starch gelatinization is an irreversible process that occurs when starch is heated in excess water, allowing the granules to absorb water and swell. Hydration of the amorphous regions within the granules will destabilize the crystalline structure and result in loss of birefringence. Starch is thus converted from a granular semi-crystalline to an amorphous form. This irreversible process causes the amylopectin double helices to dissociate, and the granules to expand as the polymers hydrate^{20,137,138}. Presence of water is crucial for gelatinization to occur^{21,139}, but other factors such as botanical origin⁹⁷, starch concentration¹³⁵, and presence of solvents^{21,140,141}.

Gelatinization of starch is an important feature to consider as swelling and/or dissolution of starch can alter the emulsification process of Pickering emulsion, as will be discussed later in chapter 5, or change the pasting and rheological properties the formulations¹⁴². Furthermore, gelatinization of the starch granules after adsorption to the oil-water interface, i.e. after emulsification, can change the barrier properties of the emulsion droplets creating an encapsulation barrier^{143–145}. This feature is interesting for the encapsulation and delivery of lipophilic ingredients.

Various techniques can be employed to study starch gelatinization and associated processes, including differential scanning calorimetry (DSC), X-ray diffraction (or both simultaneously), viscosity measurements, nuclear magnetic resonance

(NMR), Fourier transform infrared (FTIR) spectroscopy, small angle neutron scattering (SANS), or hot stage microscopy^{124,131,135,141,142,146}.

In paper III, we have used DSC and X-ray diffraction to study the gelatinization of starch in water and ethanol-water solutions, see Fig. 11. DSC measurements showed that when increasing the ethanol concentration, up to 50 wt%, ethanol had a tendency to increase the gelatinization temperature. Above 50 wt% ethanol it was not possible to determine the transition temperatures as the thermograms fell outside the measurement range (95 °C), and sample evaporation was noticed. Nevertheless, WAXS diffraction data (Fig. 11 C and D) indicate that the starch dispersions in water lose structure above 60 °C, while dispersions in 70 wt% ethanol still retained their crystalline structure.

Figure 11. X-ray diffraction data (A,B,C, and D) and DSC thermograms (E) of quinoa starch dispersions (16 wt%) in water and in ethanol-water solutions (10-50%EtOH). SAXD data displaying starch-H₂O (A) and starch-70%EtOH (B) at 25 °C (blue line), 40 °C (cyan line) and 60 °C (red line). WAXD data displaying starch-H₂O (C) and starch-70%EtOH (D) at 25 °C (blue line), 40 °C (cyan line) and 60 °C (red line). DSC thermograms of quinoa starch dispersions in 0-50 wt% ethanol (E). The data have been offset along the ordinate for clarity.

5. PICKERING EMULSIONS

Emulsions stabilized by colloidal particles are called Pickering emulsions. These are the main emulsion systems studied in this thesis (papers I, II, and III). The interfacial phenomenon behind particle-stabilized emulsions was first described by Ramsden¹⁴⁷, and later explained by Pickering¹⁴⁸ (hence the name ‘Pickering emulsion’) in the beginning of the 20th century. The recent increased interest in Pickering emulsions can be related to their high long-term stability against coalescence and Ostwald ripening, and the vast number of different types of particles that can be utilized^{37,65,149,150}. The most studied particles are silica nanoparticles^{151–154}, but other particles have also been studied such as metallic particles^{155,156}, natural clays^{157,158}, polymer particles^{159,160}, proteins^{161,162}, cellulose^{163,164}, and starch^{90,91,94,97,165}. Rising ethical and environmental demands, and the requirement of non-toxic ingredients for topical delivery motivate the search for non-toxic, biodegradable, and sustainable particles. Starch particles fall into this category and are the main particles of interest in this thesis (more specifically starch from quinoa grains) in the study of the properties of Pickering emulsions in comparison to surfactant-based emulsion.

Generally, all emulsification processes used to prepare conventional emulsions can be utilized to prepare Pickering emulsions. The most common technique used to prepare Pickering emulsions in the literature is by rotor-stator homogenization^{94,160,166,167}, but other techniques can also be used^{111,168–172}. For details on the advantages and limitations regarding emulsification techniques used for Pickering emulsions, the reader is referred to the review by Albert et al.³⁷.

Surfactants, macromolecules and particles

The stabilization mechanisms of particles in Pickering emulsions differs from stabilization by low-molecular emulsifiers (*i.e.*, surfactants), and macromolecules (*i.e.*, proteins, synthetic polymers, and polysaccharides such as molecular starch). The difference in stabilization is illustrated Fig. 12. The surfactants adsorb at the oil-water interface and reduce the surface tension, while amphiphilic macromolecules orientate in a such a way that the primarily hydrophobic parts are in the oil-phase, while the hydrophilic parts are in the water phase. In both these cases, electrostatic and steric repulsion are the main stabilizing factor. For particles however, the adsorption is driven, as will be discussed in the next section, by the size of particles and contact angle formed at the three-phase boundary where the stabilization is primarily by steric hindrance.

Figure 12. Illustration of different stabilization mechanisms. Water phase in blue, and oil-phase in orange. A. Low molecular weight surfactants ($\sim 0.4\text{-}10\text{ nm}$). B. Amphiphilic macromolecules ($2\text{-}200\text{ nm}$). C. Starch particle ($0.5\text{-}15\text{ }\mu\text{m}$).

One must be aware of the difference between emulsion stabilization with starch in granular form (*i.e.*, Pickering stabilization) and in its molecular form (dissolved starch). It is an important aspect for formulators to have in mind in the process of preparing Pickering emulsions, as heating the starch in the presence of water beyond its gelatinization temperature ($>45\text{ }^{\circ}\text{C}^{98}$) before emulsification would result in dissolution of the starch and particle stabilization cannot occur.

Stabilization mechanism for Pickering emulsions

Particles used to stabilize Pickering emulsions have to be wetted by both the dispersed and the continuous phases^{150,173,174}. The stabilizing mechanism of adsorbed particles is believed to be caused by the formation of a steric barrier of adsorbed particles between oil droplets and limiting the chance of coalescence^{174,175}. Thus, for Pickering emulsions, the relevant parameter that determines whether an emulsion will be O/W or W/O is thought to be the three-phase contact angle θ . It is the angle between the water phase, the oil phase and the particles, Fig. 13. The contact angle θ can be defined by the Young equation, Eq. 7:

$$\cos \theta = \frac{\gamma_{po} - \gamma_{pw}}{\gamma_{ow}} \quad \text{Eq. 7}$$

Where γ_{po} , γ_{pw} , γ_{ow} are the particle-oil, particle-water and oil-water interfacial tensions, respectively. In analogy to the HLB-number for surfactants, the contact angle θ can be used to determine the type of emulsion that will be realized⁴⁰. If the contact angle θ is larger than 90° a W/O emulsion will form, while a contact angle smaller than 90° will favor O/W emulsions (Fig. 13).

Figure 13. Schematic representation of an O/W and a W/O Pickering emulsion at microscopic, and nanoscopic scales. The three-phase contact angle (θ) as well as the particle-oil (γ_{po}), particle-water (γ_{pw}) and oil-water (γ_{ow}) interfacial tensions are materialized on nanoscopic scale picture (right). Reprinted from Albert et al.¹⁶⁰ ©2019 with permission from Elsevier.

When a relatively small spherical particle (so that the effect of gravity is negligible) in favorable wetting conditions adsorbs to the oil-water interface, the energy required to remove it from the interface is given by Eq 8:

$$\Delta G_{detach} = \pi R^2 \gamma_{ow} (1 - |\cos \theta|)^2 \quad \text{Eq. 8}$$

ΔG_{detach} is the free energy of detachment, R is the particle radius, and γ_{ow} is the oil-water interfacial tension^{91,150}. Eq. 8 implies that the detachment energy depends on the contact angle θ , the particle size, and the oil-water interfacial tension. In Fig. 14, the free energy of detachment divided by the thermal energy kT (k for Boltzman's constant and T for the absolute temperature) is plotted as a function of particle radius. It shows that the detachment energy increases with increasing particle size. Thus, when the contact angle θ is not far away from 90° , and the particle size is above a certain size ($R \sim 10$ nm), the adsorption of the particles at the oil-water interface will be practically irreversible due to very high detachment energy, ΔG_{detach} ^{91,150,174,176}. In comparison, small low-molecular weight surfactant molecules (< 5 nm), are in a dynamic equilibrium and generally adsorb/desorb at low energies (< 10 kT) within a relatively fast timescale^{91,150}.

Figure 14. Plot illustrating the increase in energy of detachment of a single spherical particle as a function of its size. Reprinted from Rayner et al.⁹¹©2014 with permission from Elsevier.

Contact angle measurement

There are numerous reports on different methods developed to measure the contact angle at the oil-water interface. A detailed and comprehensive review on this topic is given by Zanini & Isa¹⁷⁷. Zanini & Isa classified current methods into single-particle methods and ensemble-methods. Single-particle methods are based on direct observation of the particles or of their imprints but require large statistics and are limited to the observation of particles *in situ* with sensitivity only down to few micrometers. Sensitivity could be improved by using an additional particle-immobilization technique using SEM¹⁷⁸, AFM¹⁷⁹, cryo-SEM¹⁸⁰, and WETSEM (Wet Scanning Electron Microscopy)¹⁸¹. Ensemble-methods are based on statistical measurement and can be applied on a large range of particles, even very small particles. However, ensemble-methods normally involve assumptions regarding the interface microstructure, the particle shape at the interface, and specific adsorption/desorption mechanism^{37,177}.

In this thesis work, we have tried to apply different imaging (single-particle) techniques to observe the adsorbed starch particles at the oil-water interface. With confocal scanning microscopy (Fig. 15) we could locate the particles adsorbed at the oil droplets and quantify droplet size. However, at higher magnification the resolution was not good enough to study the shape of particles and the contact angle θ at the curved interface. Better resolution could be obtained with multiphoton microscopy¹ (Fig. 16), but it was still not sufficient enough to study particle diameters of less than 2 μm .

Furthermore, we also tried to visualize the starch particle *in situ* at the oil-water interface using synchrotron-based X-ray tomography². However, the resolution in this method was insufficient for this purpose. Nevertheless, it allowed us to study the emulsions in bulk (Fig. 17 and 18A) and when applied on skin surfaces in controlled ambient conditions (Fig. 18B). Information on behavior of formulation residue, as well as droplet size and droplet size distribution could be obtained, but it was not possible to distinguish the shape of individual starch particles. WETSEM technology and E-SEM (environmental-SEM) were also used to study the emulsions at ambient conditions but difficulties with poor contrasts and starch-gelatinization at high magnification were encountered.

¹ We would like to acknowledge Dr. Julia Fernandez-Rodriguez and Dr. Rafael Camacho at Centre for Cellular Imaging, Core Facility of the University of Gothenburg for their kind collaboration allowing us to test this technique.

² We would like to acknowledge Dr. Rajmund Mokso at MAX IV laboratory and Dr. Christian Matthias Schlepuetz at TOMCAT beamline, PSI for their collaboration and assistance.

Figure 15. Representative confocal scanning microscopy image showing oil droplets (triolein), in red, stabilized by starch particles, in green, adsorbing to the curved oil surface.

Figure 16. Representative multiphoton microscopy image showing an oil droplet of (triolein) surrounded by starch particles.

Figure 17. 3D visualization of stabilized oil droplets (triolein) in bulk of quinoa-starch stabilized Pickering emulsion using X-ray tomography.

Figure 18. Pickering emulsion with triolein visualized with X-ray tomography. A. 3D visualization of stabilized oil droplets (triolein) in bulk emulsion. B. Emulsion film when applied on excised porcine skin.

Stability of Pickering emulsions

We have already shown that small droplet size is crucial for the stability of conventional surfactant-based emulsions against creaming/sedimentation. Droplet size of Pickering emulsions are normally relatively large, ranging from few hundred nanometers to hundreds of micrometers, depending on the emulsification conditions^{37,92,94,166,167}. The large droplet size in combination with the increased weight of the droplets with particles adsorbed can result in creaming/sedimentation. However, once droplets are formed, they can be stable to coalescence for long periods of time^{65,91,94,96,182}. It has been established that for efficient stabilization particles should preferably be more than one magnitude smaller than the emulsion droplets^{159,176}. Smaller particle size reduces the ratio between amount of particles and the dispersed phase needed to stabilize a given emulsion droplet interface⁹¹, and the droplet size will be governed by this ratio and/or the emulsification technique used^{64,91,174,183}. Droplet size will decrease with increasing particle-to-dispersed phase ratio to a certain point where a plateau is reached and any further reduction can only be obtained by improving the emulsification conditions^{174,184}. Creaming/sedimentation of Pickering emulsions can be resolved by adding rheological modifiers to the continuous phase, *i.e.*, increasing the viscosity of the system, or by applying density-matching as proposed by Rayner et al.⁹⁰ to obtain buoyancy neutral emulsions without addition of rheological modifiers.

In paper I, a carbomer was added to Pickering emulsions stabilized with quinoa starch to improve the stability against sedimentation. In paper III, we could see how the fraction of dispersed phase, as well as changing the solvent of the continuous phase affect creaming/sedimentation, see Fig. 19. Nonetheless, the droplets have been seen to be stable against coalescences up to 3 years as we will show later in Fig. 20. The stability of Pickering emulsions against coalescence relies on droplets being almost fully covered by particles, forming a layer that sterically hinders coalescence. However, it has been shown that stable emulsions can be obtained even with low surface coverage¹⁷⁵, and it has been proposed that this stability is due to bridging monolayers where emulsion droplets share particles¹⁸⁵.

Particles surface roughness has been shown to increase emulsion stability to a point where it drastically decrease when the particles are no longer homogeneously wetted by the liquids¹⁸⁶. In contrast to silica and latex spheres which are often used in fundamental studies, bio-based particles such as starch granules used in this thesis, are often not hard and smooth spheres, but have a rather polyhedral topography with somewhat sharp planes. This will affect the packing density and the energy of detachment⁹¹.

Triglyceride content	41 wt%	26 wt%	18 wt%	10 wt%	5 wt%
PE TO:H ₂ O
PE TO:55wt% EtOH
PE TO:70wt% EtOH
PE TO:85wt% EtOH

Figure 19. Pickering emulsion stabilized with quinoa starch where the amount of dispersed phase (triolein) was varied in descending order (→), and increasing amount of ethanol in the continuous phase (↓). Creaming/sedimentation depends on the composition of the dispersed and continuous phases.

The effects of particle aggregation

At high particle concentrations and constant emulsification energy, there can be too many particles compared to the oil-water interfacial area and consequently there will be excess particles present in the continuous phase. If these particles flocculate, they can create a three-dimensional gel-network of interconnected particles which can improve emulsion stability by suppressing creaming/sedimentation^{65,187}. In fact, in the preparation of Pickering emulsions, particles are often pre-flocculated before emulsification, as weak flocculation is beneficial for emulsion formation^{64,96,119,187,188}. However, a high degree of flocculation can result in emulsion destabilization¹⁸⁹. Rayner et al.^{90,119} observed in two different publications that aggregation of particles causes droplets to aggregate, and that increasing the hydrophobicity of particles increases the inter-particle aggregation¹¹⁹. Furthermore, the same authors reported that increasing the hydrophobicity beyond a certain threshold value led to increase in droplet size. In paper III (Fig. 20), we could see a clear difference in degree of

aggregation in emulsions with water as the continuous phase in contrast to mixed ethanol-water emulsions. We also noted a tendency of larger droplets being formed when ethanol was used in the continuous phase. This, in combination with density differences between water and ethanol-water solutions could explain the difference in sediment/creaming layer for pure water contra ethanol-water emulsions seen in Fig. 19.

Figure 20. Micrographs (20 x magnification) showing oil droplets in Pickering emulsions with 3:7 ratio of dispersed phase to continuous phase for triolein (A-C), and tricaprins (D-F). Triolein emulsions with water (A, droplet size $\sim 20\text{-}35\ \mu\text{m}$), with 55 wt% ethanol (B, droplet size $\sim 30\text{-}50\ \mu\text{m}$), and 70 wt% ethanol (C, droplet size $\sim 30\text{-}50\ \mu\text{m}$). Tricaprins emulsions with water (D, droplet size $\sim 25\text{-}50\ \mu\text{m}$), with 55 wt% ethanol (E, droplet size $\sim 40\text{-}70\ \mu\text{m}$), and 70 wt% (F, droplet size $\sim 50\text{-}90\ \mu\text{m}$). The scale bar is $50\ \mu\text{m}$.

The presence of co-emulsifiers

Despite the high surface energy required to detach particles from the oil-water interface of droplets, there have been reports of particles displaced from the interface by adding surfactants^{190–194}. It is argued that this can happen either by surfactants adsorbing to the particle surfaces and modify their wettability *in situ*^{174,191,192}, or as a competitive displacement where the surfactants compete with the particles to adsorb at the oil-water interface^{174,190}. Other studies have focused on studying synergies between particles and surfactants^{195–197}. However, it can be argued that the addition of surfactants to a Pickering emulsion counteracts the attractive benefits of using such systems to replace surfactants. In general, we have experienced destabilization of Pickering emulsions in the presence of emulsifiers, surface-active polymers, surface-active preservatives. Furthermore, surface-active components may be present in other excipients, and it is therefore important to consider the functionality and purity of other excipients when formulating Pickering emulsions.

Alcohol-based Pickering emulsions

The effects of using ethanol-water mixtures as the continuous phase of an O/W Pickering was studied in Paper III. Stable alcohol-based Pickering emulsions were successfully produced, and the emulsions were stable against coalescence and Ostwald ripening even after 3 years (Fig. 21). These emulsions were produced as a possible moisturizing alcohol-based hand rub.

Figure 21. Micrographs showing the emulsions after > 3 years storage at room temperature. Triolein (A) and tricaprin (B) Pickering emulsions with 3:7 ratio of dispersed to continuous phase. Photographs of corresponding bulk emulsion in glass containers are attached. A: Triolein Pickering emulsions with 70 wt% ethanol with droplet size ~ 20-50 μm . B: Triolein Pickering emulsions with 70 wt% ethanol with large droplets ~ 70-100 μm . The scale bar is 50 μm .

The antimicrobial efficacy of Pickering emulsions has been tested according to EN 13727¹⁹⁸ and published as part of our related patent application (Paper VIII)¹⁹⁹, see Table 1. The alcohol-based Pickering emulsions comprised 26 wt% triolein as the dispersed oil phase, and 35 wt% (Pickering 35% EtOH) and 42 wt% ethanol (Pickering 42% EtOH), respectively. A water-based Pickering emulsion (Pickering 0% EtOH) with the same oil content was included as a control. The Pickering emulsions were also compared to ethanol solutions comprising 35 wt% (35% EtOH (aq)), 42 wt% (42% EtOH (aq)) and 58 wt% (58% EtOH (aq)) ethanol in water.

The test showed that alcohol-based Pickering emulsions with at least 35 wt% ethanol (Pickering 35% EtOH) are effective against both *E.coli* (*Escherichia coli* K12) and *S.aureus* (*Staphylococcus aureus*), while ethanol solutions comprising 35 wt% and 42 wt% ethanol were not effective against *S.aureus*. It was not surprising to see that the water-based Pickering emulsion lacks antimicrobial efficacy. This indicates that the presence of starch and triglycerides in the alcohol-based Pickering emulsions did not contribute to the antimicrobial efficacy of the ethanol solution in the continuous phase.

Table 1. Antimicrobial efficacy of Pickering emulsions compared to aqueous ethanol solutions. The Pickering emulsions comprised 26 wt% oil dispersed in an aqueous continuous phase with increasing ethanol content. Method EN 13727 (Bactericidal efficacy in suspension test, dilution to 80%, $\log_{10} \text{RF} \geq 5$), comprising *Escherichia coli* K12 and *Staphylococcus aureus*. The efficacy is noted with Y (yes) or N (no).

Formulation	E. coli / $\log_{10} \text{RF}$		S. aureus / $\log_{10} \text{RF}$	
	80%	Effective (Y/N)	80%	Effective (Y/N)
58% EtOH (aq)	> 5.4	Y	> 5.3	Y
42% EtOH (aq)	6.4	Y	4.8	N
35% EtOH (aq)	> 5.4	Y	3	N
Pickering 42% EtOH	6.3	Y	6.4	Y
Pickering 35% EtOH	> 5.4	Y	> 5.3	Y
Pickering 0% EtOH	< 2.7	N	< 2.6	N

The work in paper III shows that it is possible to develop an ethanol-based Pickering hand rub with cosmetically appealing properties. This was concluded by tactile friction measurements and tactile perception by a human panel, where an ethanol-based Pickering emulsion with 5 wt% triolein was the most preferred and ranked highly over a commercial hand sanitizer, specifically for afterfeel attributes.

6. CHARACTERIZATION OF FORMULATIONS

The evaluation and characterization of topical formulations and excipients was performed using several well-established characterization techniques. These are shown in Table 2 with comments on why they were used. Solutions were characterized to evaluate their water activity and the solubility of the API in the formulation. During the development of topical formulations, excipient physicochemical properties were evaluated. Once emulsions or creams were prepared, they were evaluated for macroscopic appearance regarding creaming or sedimentation. The emulsions were further evaluated using microscopy techniques (*light*, *confocal*, *SEM*), and in some cases laser diffraction particle size analyzer (Malvern Mastersizer S, Malvern instruments, UK) to confirm particle stabilization and to determine droplet size. Laser diffraction is a useful technique to determine the particle- and droplet size distribution in the sample (0.01-3500 μm). However, the mixing speed of the sample dispersion unit, and particle- or droplet aggregation may give inconclusive results, *e.g.*, overestimation of the droplet sizes. Since the particle and droplet sizes are in the micron range, microscopy techniques were widely used to study and follow emulsion stability.

The flow properties and rheological characteristics of the formulations can be studied with a rheometer. In paper I, we link the measurements of physical properties, including rheology, to the sensory properties of creams as measured by an untrained panel. Rheological measurement values were seen to correlate with application-related attributes such as thickness and spreadability. Finally, as discussed later in this chapter and in chapter 3, the *in vitro*, *ex vivo*, and human appreciations of the formulations were evaluated.

Table 2. An overview of the methods used in this thesis.

Method	Purpose	Paper
Analytical methods		
HPLC-UV	Determination of drug content in experiments related to solubility measurements and <i>in vitro</i> and <i>ex vivo</i> penetration studies	IV, V
UV spectroscopy	In some cases the in-vitro penetration studies could be conducted using the easier method of UV spectroscopy	IV
Automatic gamma counter	To determine low concentrations of a Cr-EDTA radioactive labeled substance, the method was used in ex-vivo penetration studies	V
Thermogravimetical Analysis	Determination of triglyceride content for phase diagrams	III
Physico-chemical characterization methods		
Water-activity measurements	To determine water activity of formulations used in penetration studies	IV, V
Rheology, viscosity	Investigation of flow properties	I
Rheology, oscillation	Investigation of structural properties	I
Light microscopy	To confirm particle-stabilization, determine droplet size and aggregation behavior	I, II, III
Static light scattering	To determine particle and droplet size distributions	Thesis
SEM	To visualize microstructure of particles, substrate structure, and residual film structure	II, III
WETSEM	To visualize the microstructure of formulation (contact-angle)	Thesis
Confocal Scanning Microscopy	To visualize the microstructure of formulation (contact-angle)	Thesis
Multiphoton Microscopy	To visualize the microstructure of formulation (contact-angle)	Thesis
Synchrotron X-ray tomography	To visualize the microstructure of formulation dependent behavior of residual films	Thesis
DSC	Thermotropic behavior of starch and lipids	III
X-ray diffraction	Crystal structure of starch and lipids	III

Method	Purpose	Paper
In-vitro methods		
Permeation studies using Franz cells	To evaluate drug permeation across synthetic membranes and thus formulation features	IV, V
Permeation studies using flow through cells	To evaluate drug permeation across synthetic membranes and thus formulation features	IV, V
Tactile friction	Measuring the friction coefficient of VitroSkin® when applying a formulation.	II, III
Ex-vivo methods		
Diffusion measurements using Franz cells	To evaluate drug permeation across topical membranes and thus the effect of the formulation on the barrier	IV, V
Diffusion measurements using flow through cells	To evaluate drug permeation across topical membranes and thus the effect of the formulation on the barrier	IV, V
Skin hydration, by electrical capacitance	To measure skin moisture based on capacitance	I, II, III
Tactile friction	Measuring friction coefficient of excised skin when applying a formulation.	I, II, III
Methods using human research subjects		
Human sensory panel	Evaluating and ranking cosmetic properties of the formulations	I, III
Digital survey	To understand the preferences of the human sensory panel in the current studies	I, III
TEWL	Measuring endogenous water loss	III
Skin hydration, by electrical conductance	To measure skin moisture based on conductance	I, II, III

Tactile perception

Sensory study by a human panel

Tactile perception as measured by a human sensory panel is often used in the cosmetic and pharmaceutical industries to evaluate the texture and feeling of topical formulations during the application, as well as the afterfeel after spreading and evaporation of the volatile compounds. Preference and descriptive sensory tests are quite complicated to conduct and can require a well-trained panel and proper choice of terminology^{200,201}. Such studies are time-consuming, costly, and are often subjective^{70,201}. A consumer panel does not require training and is normally used for hedonic preference and are useful when pre-sorting samples in a preliminary test²⁰². However, to account for the large variability, a larger number of panelists are required^{201,203}. The test objectives, time and budget considerations often determine if trained panelist or naïve/consumer panelists are to be used^{200,201}. In this thesis we have used a sensory study based on the ranking test^{200,202,204}. It is a suitable test to compare several samples according to a single attribute. By ranking the samples, the data obtained will be ordinal, and no measure of the degree of difference or preference can be obtained from each assessor^{200,201}.

Considerations when designing a sensory study

Setting up the sensory tests required a lot of considerations and planning, several aspects of which are mentioned below. For the exact protocol used in each study, the reader is referred to paper I and paper III.

The first thing that needs to be considered when planning a sensory study is what is to be measured and what is the test objective? The safety of the samples and ingredients needs to be assured, and the requirement for ethical permission checked. Other considerations include the kind of test to be conducted? Who is taking part in the study? How many assessors are needed?

The recruitment of assessors also involved communicating the test objective and the test procedure. The safety of the assessors had to be considered, and thus in our studies individuals with a history of skin disease or allergy were excluded. To avoid biases, people with prior knowledge about the samples tested, or were involved in the project were also excluded.

Since we used a naïve panel, with no experience of sensory testing, the method had to be standardized and set-up in a way to minimize the risk of confusion and bias. Among the things considered was the order of presenting of samples, randomization, coding, ease of distinction, and ease of using the survey.

Before starting the test, the assessors were introduced to the terminology used for each attribute with demonstration of the instructions on how to assess each attribute.

Tactile friction

Tactile perception is a complex combination of sensory stimuli including skin stretching, pressure applied, and vibrations during touching²⁰⁵, and is an elusive sensation to capture. When a finger slides on a sensed surface, the applied load needed to slide the finger is determined by the tactile perception (perceived touch) and the resulting measurable friction that develops between the finger and surface can be referred to as the tactile friction (see Eq. 9 below).

When surfaces are brought into contact, and sliding over each other, a force will develop in the opposite direction relative to the movement between the two surfaces. This force is called the friction force (F), and is described by the friction coefficient (μ) which is defined as the ratio of friction force (F) and the applied load (L)^{206,207}:

$$\mu = \frac{F}{L} \quad \text{Eq. 9}$$

Tactile friction has been used to study effects of humidity^{70,208–210}, treatment time and different substrates used²⁰⁵, hydration^{211,212}, occlusive components²¹⁰, oil content⁴⁹, and particles present²¹³. In particular, hydration of skin has been related to higher friction^{211,212}, while greasiness and slipperiness to lower friction⁴⁹. Variation between different subjects performing friction measurements were found to provide similar trends in friction, meaning that friction results from one individual can be representative of a larger group if relative comparisons in friction are used. However, comparison of absolute friction coefficient values between two different experimenters should be avoided²⁰⁷.

Techniques that have been employed to measure skin friction, include a sliding^{212,214} or rotating probe^{49,60}, an artificial finger^{70,205}, and sliding a human index finger on surfaces attached to a friction board (Forceboard™)^{208,210,215,216}. For a more detailed description of tactile friction the reader is referred to the review by Derler²¹⁷ and Guest et al.²¹⁸ and the comprehensive doctoral thesis by Skedung²⁰⁷.

The finger friction method developed by Skedung et al.,²¹⁰ has been adapted in this thesis and modified to study and compare topical formulation on excised porcine skin (paper I and II) and VitroSkin® using a Forceboard™ (Industrial Dynamics AB). The Forceboard™ measurements are described in detail in paper II, and the setup is illustrated in Fig. 22. The Forceboard™ is equipped with two load cells, one horizontal

and one tangential, consisting of strain gauges in a Wheatstone bridge configuration. A mechanical load is converted into voltage signals that are amplified and proportional to the applied load. Forces are sampled at 100 Hz using DAQFactory software²⁰⁷.

Figure 22. Photograph of the author (experimenter one) performing tactile friction experiments and substrates attached on the heating plate on top of the ForceBoard™ with corresponding SEM image when untreated. On the left: excised porcine skin. On the right: VitroSkin®. Scale-bar 20 μ m.

In paper II, the tactile friction of topical formulations applied to excised skin and VitroSkin® was evaluated. The purpose was to evaluate whether VitroSkin® gives results similar to excised skin and if it can be used as a substitute. That would allow quick preparation, better reproducibility (compared to biological variance in excised skin) and avoiding the use of animal or human skin. The friction coefficient resulting from sliding the index finger of one experimenter on untreated VitroSkin® and excised skin was compared. Similar friction coefficient values were obtained, but larger variation was seen for excised skin. We further show that absolute friction coefficient values measured on untreated substrates differed between two experimenters. To study repeated measurements over time, we have proposed normalization of the friction coefficients against the initial friction coefficient of untreated substrates. When topical formulations were applied the difference in normalized friction was small between two experimenters. It was concluded that to perform comparisons of exact values of friction coefficients the same experimenter should be used during the full study.

Understanding friction during spreading

In paper I, II and II we have used this technique to compare different formulations and their behavior over time. To understand the results, it is important to understand different lubrication regimes, and this is described in Fig 23.

The process of lubrication when stroking a finger on a body site or surface with a fluid layer over time has been divided into three main lubrication regimes, *i.e.*, the “boundary”, “mixed”, and “hydrodynamic” regime ²¹⁸. The appearance of these lubrication regimes is believed to depend on the characteristics of the applied cream and the thickness of the formed film. When the skin is untreated, it can be imagined that the friction coefficient is affected by the characteristics of the fingertip and skin surface in the “boundary region”. When a topical formulation is applied, the film between the finger and the substrate is thick, and the friction coefficient is mainly influenced by the formulation viscosity and thickness, and hence the effects from skin texture are minor during hydrodynamic lubrication. Subsequent spreading of the formulation and evaporation of volatile compounds leads to a residual thin film. At this stage, mixed lubrication may occur leading to a moderate increase in friction as result of the changing properties of the residue and/or the underlying surface. It has indeed been shown that hydration of the surface by an aqueous vehicle or by occluding effects can increase friction over time ^{49,70}. When the thickness of the residual film is reduced, lubrication resembles the boundary regime and the friction will again be dominated by the surface properties of the skin ^{70,218}.

Figure 23. The lubrication regimes that may occur during touch of surfaces with a fluid film spread between finger and skin or other surface. Reprinted from Guest et al.²¹⁸

In paper II, we observed that by the end of the measurement (11 minutes), formulation residues could still be detected on top of excised skin and VitroSkin® and the two substrates were in the mixed/boundary region. This means that the friction will be affected by both the formulation residual film and the texture of the substrate.

When topical formulations were applied to excised skin and VitroSkin®, in paper II, the two substrates showed similar trends in results but differed in the absolute values and friction changes over time. These differences were attributed to the different surface texture of the two substrates (see SEM images in Fig. 22) but also to differences in formulation properties, Pickering formulations in particular. The effect

Figure 24. Normalized friction coefficient of applied Pickering emulsions with varying triolein oil content (and starch concentration 300 mg/g oil) on excised skin and VitroSkin®: 5 wt% (light grey bar with white texture) and 26 wt% oil (black bar with white texture). The tests with Pickering cream (emulsion with other excipients) with 28 wt% oil (white spheres) are also included for comparison.

on tactile friction related to emollient and particle content in Pickering emulsions were also studied on excised skin and VitroSkin® (Fig. 24). The results show that once the oil has been rubbed in on excised skin, the structure of the residual film of Pickering emulsions is similar irrespective of oil content. However, on VitroSkin® when higher oil content is used (26 wt%) the difference in friction change was much higher as a result of higher sensitivity for the presence of particles. This was attributed to the difference in topography between excised skin and VitroSkin®, where the latter lacks the ability to absorb the oil and the residual film becomes like a paste of starch and oil.

Relating physical and sensory properties

The properties of the formulation during the application phase are often considered important for the first perception of the formulation by consumers. During the application, the topical formulation needs to spread easily without feeling too greasy⁷⁸, or grainy. Therefore, the sensorial attributes of the formulation are very important.

Several authors have tried combined approaches to connect physical characteristics of topical formulations to sensory attributes^{48–50,60,70,210,219–222}. It would allow combining subjective and objective methods to obtain effective and accurate studies of tactile perception⁷⁰. Using physical methods to assess the sensory properties of topical formulations allows for fast prototype screening in the early stage of formulation design. Rheology^{48,50,219,223–225}, tactile friction^{49,60,70,210,213,222} and skin hydration^{60,219} are among the methods that has been previously correlated to sensory attributes, and which have been employed in this thesis. However, none of these studies have compared the sensorial, and physical properties of surfactant-based and Pickering-stabilized creams.

Based on the information gathered in the questionnaire from the human sensory panel, it was obvious that several different aspects of the cream were important for choosing the product most preferred. Among the aspects, the afterfeel of the formulation is often preferred to be non-greasy and soft. In order to design topical formulations with such a complex sensorial composition, there is a need for tools that link sensorial attributes to more objective and easily performed measurements. This will enable the formulator to identify excipients that are important when designing a formulation with specific physical and sensorial properties.

In paper I, we studied the rheological flow and structural properties, the hydration effect, and tactile friction of four different creams, and linked these results to sensorial

Figure 25. A summary of physical and sensorial results obtained from four different creams studied in paper I. A. Rheological flow properties. B. Rank sums of perceived attributes by human panel. C. Normalized tactile friction. D. Relative change in hydration *in vivo*. The formulations were Pickering cream in green, surfactant-based cream replica of Pickering cream in blue, surfactant-based cream in yellow, and surfactant-based commercial cream in purple.

attributes as perceived by a human panel. The formulations were one Pickering-stabilized cream and three surfactant-based creams, *i.e.*, a commercial product (Canoderm®), a surfactant-based replica of the Pickering-cream, and a simple surfactant-based cream with common occlusives. For the exact formulation compositions, the reader is referred to paper I (Table 1). In Fig. 25, some of the results from this study are highlighted, such as the rheological properties, tactile perception by ranking, tactile friction data, and *in vivo* hydration data.

In summary, these results indicated that there was a large difference in physical and sensorial properties of the Pickering cream and surfactant-based creams, but there were also some differences among the surfactant-based creams. It was also possible to relate some of these differences in physical properties to the tactile perception of certain sensorial attributes. To resolve these differences and relate the different physical and sensorial properties to specific excipients we conducted partial least

square (PLS) as a visual tool to link sensory properties and physical chemical properties to the four creams studied. The PLS (Fig. 26) is interpreted in detail in paper I. The four studied creams were distributed quite well in both dimensions of the PLS, indicating that each cream appears to have unique characteristics in their perceived attributes. Surfactant-based creams differed from each other along the y-axis from ‘thick’ to ‘moist’, mainly identifiable by differences in hydration and rheological data, linked in turn to emollient and thickener composition. On the other hand, the Pickering cream differed from surfactant-based creams along the x-axis, which was primarily related to afterfeel sensory attributes and tactile friction. This was probably related to the presence of starch particles in the residual film. The present results, as visualized by PLS, suggest that tactile friction experiments could be used to determine sensorial attributes related to afterfeel, and rheological experiments could be used to determine attributes relating to thickness, and spreadability. Sensory and physical characterization was also used in paper III, where it was observed that the human panel could discriminate between the amount of emollient and particles used. The examples shown here demonstrate that it is possible to link tactile sensory attributes to measurements of physical assets such as tactile friction and rheological quantities.

Figure 26. Partial least square (PLS) visualizing the relationships between sensory data (circles), physical data (black stars) and studied creams. Sensory data are shown as attributes studied during the application phases (white circles) and during the afterfeel phase (black circles). The studied creams are Pickering cream (green circle), Surfactant cream (yellow diamond), Pickering replica surfactant cream (blue triangle), and Canoderm® (purple square).

7. CONCLUSIONS

This thesis covers a broad scope of topics concerning topical delivery and formulation. The more detailed conclusions are described in the individual papers. In this section the most important and novel conclusions are highlighted.

In paper I we show by relating physical chemical characterization with sensory evaluation how a deeper understanding of how formulation related factors influence the consumer appeal of topical formulations can be arrived at. In particular, we conclude that starch-based Pickering emulsions are perceived as less greasy and sticky than traditional creams, even at high oil content. The difference between a Pickering cream and surfactant-based creams was more evident in relation to the afterfeel perception, which was linked to tactile friction measurements.

This work has also shown that a naïve human panel, using a ranking test, can discriminate between different emulsion types, emollient content, and the particle content of a formulation.

In this thesis we have developed the *in vitro* ForceBoard™ method further and evaluated its possibilities to be used as an *ex vivo* method. The topography of excised skin highlighted formulation related aspects that were not seen before with an artificial substrate such as VitroSkin®.

By combining results of tactile friction, and SEM we were able to show that the reason that the human panel identified the afterfeel of a Pickering emulsion as less greasy was related to the microstructure of the residual film containing starch and residual oil.

We have also been able to successfully formulate an innovative alcohol-based Pickering emulsion that can be used as a moisturizing hand rub. Changing the solvent composition of the continuous phase from water to ethanol was found to affect physical properties, *e.g.*, emulsion droplet size.

Previous studies did show that local dehydration by a low water activity formulation decreases the drug permeability of skin. In this thesis we showed that this in fact has an extended biologic relevance and is applicable for both buccal and nasal mucosa.

More importantly, in this thesis we have emphasized the importance of water in topical formulation and across barrier delivery. The work has highlighted the role of water from recipe formulation, to application of a formulation, payload penetration,

and final uptake. In the recipe, water is important for the hydration of several excipients, and water governs the formulation type obtained within the pharmaceutical triangle. During the application phase water affects sensory perceptions, and the reconstitution of the formulation at the biological target. Regarding uptake, the water gradient will influence the barrier properties and permeability. As shown in this thesis, this can be controlled by varying the water activity, the degree of occlusion, or by addition of humectants.

8. FUTURE PERSPECTIVES

Formulation considerations on topical drug transport

During this work we have seen several features that make Pickering emulsions interesting for dermal drug delivery. By employing the methods developed in our group we can understand how the diffusion of active substances through topical membranes is affected by Pickering emulsions, and appreciate the effect the formulation has on the skin, compared to release from the formulation.

The droplets of Pickering emulsions can be quite intact and stable, and often require shear force (by spreading) or evaporation of the volatile compounds in the continuous phase to break. Even so, when the water or ethanol evaporates from the formulation, the residual film is formed in two stages:

1. By coalescence of the oil droplets with starch forming a boundary network around the deformed droplets (Fig. 27)
2. Complete collapse of the oil droplets forming a continuous oil film containing starch.

It would be interesting to utilize this feature to investigate drug delivery from Pickering emulsions with rapidly evaporating solvents, *i.e.*, ethanol, in the continuous phase to supersaturate the API, and create a concentration gradient to enhance drug transport. However, the choice of treatments is important in this case as ethanol can be perceived as stinging or cause pain when applied to open wounds or dry skin.

Figure 27. Confocal micrographs showing the starch network formation around the oil droplets before complete breakage of the oil droplets. Scale bar 25 μm.

Other formulation considerations

Ethanol-based Pickering emulsions are not only interesting for dermal drug delivery and as antiseptic emulsions. The cooling sensation combined with the non-greasy afterfeel are interesting from a cosmetic point of view as well. Light lotions or sunscreens can be designed for quick reformulation after application to moisturize the skin and leave a fresh and non-greasy afterfeel.

Method considerations

The effect of ethanol and reduced water content on the gelatinization of quinoa starch is also interesting to study further to understand the effect of starch concentration as well as water content on the gelatinization temperature of starch.

Accurate measurement of the *in-situ* contact angle of starch particles when attached to oil droplets would be very useful to study:

- a) the type of emulsions, (O/W) or (W/O), that can be produced with quinoa starch.
- b) how solvent changes of the continuous phase modulates the oil-water-particle interface.

ACKNOWLEDGEMENTS

First, I would like to express my sincere gratitude to my supervisors **Johan Engblom** and **Marie Wahlgren**. Without your enormous support and encouragement this thesis would not be in place today. **Johan**, thank you for all the support through-out the years. You have always been helpful and attentive to my thoughts and ideas and most importantly, you have helped me grow a lot as a scientist. Your passion for science and your creative ideas to merge science and industry together have been inspiring to me. **Marie**, thank you for always encouraging me and guiding me back to the right path whenever I get lost. I have always admired your ability to make even the most complicated things seem simple. Thank you both for handling me during the last stressful days, offering me your time and support despite vacations and late hours.

I would also like to thank, **Malin Sjöö**, for allowing to combine academia with industry at Speximo. For introducing me to Pickering emulsions and teaching me all about starch, and for always trusting and believing in me, and offering support whenever needed. It was a pleasure to work with you at Speximo.

I would like to thank all my co-authors and collaborators for all the scientific discussions and technical assistance. Special thanks to **Vitaly Kocherbitov** and **Chris Andersson** for in-depth contributions, and to **Lovisa Ringstad** and **Lisa Skedung** for introducing me to tactile friction and for the fruitful collaboration and technical-assistance.

I would like to show my deepest gratitude to **Zoltan Blum** for quick and effective proofreading of my papers and this thesis and for being supportive and flexible.

I would also like to thank my colleagues at Malmö University, Speximo and the French team at Lucas Meyer Cosmetics for making this journey as an industrial PhD-student so productive and memorable.

To **Sofia** and **Pilar**, for great teamwork at Speximo and for all creative meetings and joyful chats.

To **Emelie**, **Tania** and **Yana**, for your friendship and the scientific discussions, and for making conferences abroad a fun experience.

To **Max, Hannah, Sarah** for early but “always late” well-needed morning workouts. **Max**, thank you for the company and discussions on the courses we took together. To **Skaidre** for interesting discussions. To **Sabrina** and **Enamul** for the scientific collaboration and friendship. You have all made life as a PhD-student joyful, with a lot of Sweets and Cakes!

Special thanks to **Peter Falkman** for the great SEM-images and for always fixing whatever needs to be fixed in the lab, and to **Emelie** and **Juan Fransisco Gonzales** for helping with the SAXS-experiments.

I am most grateful to my parents **Majedah** and **Ali** for always being my motivation, and for the limitless support I’ve been given throughout the years. To my sisters **Heba** and **Amna** for all the encouragement and to my brother **Abdul** for assisting with the front cover. To my parents-in-law, and especially my father-in-law **Hameed** for proofreading my Arabic summary.

To my dear kids **Thalya** and **Noah**, for making every morning stress-relieving and lovely with your adorable smiles.

Finally, my deepest gratitude and love to the one person who gave me the strength to finish this thesis, to my **Zahraa**. Without your endless encouragement, support and understanding, this would not have been possible. When it is your turn to defend your thesis, I will be there for you.

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I

Relationship between sensorial and physical characteristics of topical creams: *A comparative study on effects of excipients*

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Key words: Surfactants, Emulsifiers, Emollients, Thickeners, Creams, Pickering Emulsions, Tactile friction, Sensory panel, *In vivo* hydration.

Abstract

Key formulation excipients can affect cosmetic properties of topical creams. This could be demonstrated through sensory analysis using a human sensory panel, in combination with characterization of physical properties of the creams. The aim of this paper is to study possible relationships between physical properties and sensorial characteristics and understand how different excipients affect these aspects, with particular emphasis on traditional surfactant-based creams and starch-stabilized Pickering creams. In this paper we compare, in a range of different topical creams, sensorial perception by a human panel and physical properties of the creams using tactile friction and rheology, in combination with studying effects on skin hydration.

The results show that sensorial attributes related to the application phase and afterfeel sensation of creams can be predicted by means of rheological and tactile friction studies, respectively. Difference in rheological and sensory results among surfactant-based creams could mainly be attributed to type of emollient used, thickeners present, and surfactant composition. Differences between a Pickering cream and surfactant-based creams were more evident in relation to the afterfeel perception, and well discriminated by tactile friction measurements. Application of a starch-based Pickering cream on skin results in high tactile friction and low perception of residual coating, stickiness, greasiness, and slipperiness in sensorial afterfeel.

1. Introduction

Development of cosmetically appealing topical formulations is important for both the cosmetic and pharmaceutical industry. Topical drug formulations are used for local treatment of various types of skin disorders but are also interesting for systemic delivery. The main advantage of topical delivery is avoidance of the hepatic first-pass metabolism, allowing local skin treatment, and decreasing the risks of side effects from some drug substances. Emulsions in the form of creams, lotions, gels or foams are the most common formulations for topical delivery as they have good cosmetic properties and are suitable for delivery of hydrophobic substances (Marto et al., 2016; Wahlgren et al., 2013).

There is a constant demand for formulators to develop novel safe formulations of already existing patented drug substances (Wahlgren et al., 2013). Formulations need to meet constantly shifting consumer demands in terms of patient-compliance, cosmetic appeal (Lee and Maibach, 2006) and rising ethical demands by consumers (*e.g.* natural and sustainable), while still retaining the function intended. New formulation prototypes need to be analyzed for functional as well as cosmetic properties. Functional properties can be studied by physical measurements, while the cosmetic and sensory properties of topical products are normally evaluated by human panels. Panel-based sensory studies normally require a trained group of panelists or a large consumer panel and can be time consuming and costly. Furthermore, human panels provide subjective evaluation of the products that is not always beneficial. The use of physical methods to screen new formulation prototypes for certain sensory attributes would be more suitable, allowing for more objective evaluations and major savings on time and costs. In addition, understanding how specific ingredients affect the physical and cosmetic properties relating to certain sensory attributes would be useful to help formulators develop products that meet consumer demands. Thus, there is a need to find physical methods which correlate with specific sensory attributes, and that can distinguish ingredients that cause a certain perceptible sensation. Rheological measurements (Brummer and Godersky, 1999; Gilbert et al., 2013; Greenaway, 2010; Lukic et al., 2012; Parente et al., 2005; Wang et al., 1999) and friction analysis (Lodén et al., 1992; Nacht et al., 1981; Skedung et al., 2016; Timm et al., 2012) previously have been evaluated to predict certain sensory attributes. Rheological measurements were found to be a useful tool to evaluate sensory attributes of cosmetic emulsions (Gilbert et al., 2013) and tactile friction measurements have been shown to help obtain an objective understanding of some consumer perceptible sensory attributes (Lodén et al., 1992; Nacht et al., 1981; Skedung et al., 2016).

The properties of topical formulations can be varied a lot by just changing the ratio between water and oil. The amount of water in a topical formulation affects both the skin feel during application and the afterfeel of the formulation on the skin surface as the water evaporates. Emollients can vary in molecular and chemical structure affecting their melting point, polarity, and viscosity. Emollient type has been shown to affect skin feel, skin friction, and spreadability of the topical formulations. Furthermore, emollients can also induce occlusive effects which hydrate the skin by lowering transepidermal water loss (TEWL) (Gore et al., 2018; Greenaway, 2010; Nacht et al., 1981; Parente et al., 2005; Rawlings et al., 2004; Shai et al., 2009). Humectants are hydrophilic compounds with the capacity to retain skin water, thus maintaining

hydration and minimizing water loss (Albèr et al., 2013; Lodén M, 2003; Tang et al., 2015). The stability of formulations can be improved by addition of rheological modifiers that adds body and thickness, and cosmetic powders are commonly added to the formulations to improve skin feel during and after application (Moussour et al., 2016; Timm et al., 2012; Wang et al., 1999). Synthetic surfactants are normally used to stabilize emulsion droplets, but they could be toxic or irritating especially to patients with sensitive skin (Berardesca et al., 2013; Lodén et al., 1992; Lu and Moore, 2012; Veenstra et al., 2009; Wibbertmann et al., 2011). Particle-stabilized emulsions, so called Pickering emulsions, have gained a lot of interest recently, partially related to the above-mentioned formulation demands such as producing a mild surfactant-free product, and the possibility to use natural ingredients, and create new textures. But the main interest in Pickering emulsions is related to the fact that these emulsions can have high long-term stability towards coalescence and Ostwald ripening (Timgren et al., 2013). The addition of particles to surfactant based formulations and aqueous suspensions (Moussour et al., 2016; Timm et al., 2012), as well as Pickering topical formulations (Marku et al., 2012; Marto et al., 2015) has been studied before. However, none of these studies have compared the sensorial and physical properties of surfactant-based creams and surfactant-free Pickering creams.

In this paper we aim to compare, in a range of different topical creams, sensorial perception by a human panel with measurable physical properties of creams with tactile friction and rheology, in combination with studying effects on skin hydration. The aim is to study possible relationships between physical properties and sensorial characteristics and to appreciate the effect of different excipients on the interplay, with particular emphasis on traditional surfactant-based creams and starch-stabilized Pickering creams. Specifically, we would like to investigate how presence of Pickering particles, surfactants, and alternative emollients affect sensorial properties during application and also the afterfeel of the residual film.

2. Material & Methods

2.1 Materials

Modified quinoa starch with 0.5 – 3 μm diameter granules (mean diameter = 1.7 μm), kindly provided by Speximo AB (Lund, Sweden), was used as stabilizing particles. The starch was hydrophobically modified with octenyl succinic anhydride (OSA) (Timgren et al., 2013). The emollients used were medium chained triglyceride (MCT) (Miglyol 812N, IOI Oleochemical, Hamburg, Germany), isotridecyl isononanoate (Crodamol TN, Croda, East Yorkshire, England), dimethicone (BRB DM 350, BRB International BV, Thorn, The Netherlands), hydrogenated coco-glyceride and canola oil (Akosoft 36 and Lipex Preact, AAK, Karlshamn, Sweden), jojoba oil (Natura-Tec, Fréjus, France). Liquid paraffin oil was purchased from Sigma-Aldrich (Stockholm, Sweden).

The surfactants used for surfactant-based formulations were cetearyl alcohol (Nafol 1618H, Sasol Performance Chemicals, Hamburg, Germany), PEG-100 stearate (Myrj S100), and glyceryl stearate (Cithrol GMS 40) obtained from Croda Europe (East Yorkshire, England).

Other functional excipients were Carbopol Ultrez 30 (Lubrizon, Brussels, Belgium) (also called carbomer), used as a rheological modifier and tocopheryl acetate (Dermofeel E74, Evonik Dr Straetmans, Hamburg, Germany) was used as an antioxidant. Glycerol (Sigma-Aldrich, Stockholm, Sweden) was used as a humectant and the preservatives used were phenoxyethanol and caprylyl glycol (Versatil PC, Evonik Dr Straetmans, Hamburg, Germany), propyl-4-hydroxybenzoate (Propyl paraben, Solbrol P) and methyl-4-hydroxybenzoate (Methyl paraben, Solbrol M) were provided by Lanxess GmbH (Leverkusen, Germany). Milli-Q water, 18.2 M Ω cm resistivity, was used for all samples.

2.2 Formulations

All the formulations studied in this work were oil-in-water (O/W) emulsions. A commercial surfactant-based pharmaceutical cream was included (Canoderm[®]) for comparison, purchased from a local pharmacy in Sweden. The creams prepared in the lab (batch size ~100 g) were a surfactant-free Pickering cream with carbomer (PC.c), a surfactant-based cream (SC.c) with carbomer and equivalent composition to PC.c, and a surfactant-based cream (SC) without a thickener and with different emollient types. The compositions of the cream formulations are given in Table 1.

The PC.c was prepared by mixing the water-soluble and oil-soluble excipients separately. The oil phase was heated to 60 °C until all emollients were melted, and the temperature was brought down to < 45 °C. The starch was added to the water phase below 45 °C, during mixing with a propeller stirrer (IKA RW-20, IKA, Germany), mixed for additional 5-10 minutes before slowly adding the oil-phase with stirring. The mixture was stirred for an additional 5-10 minutes before emulsification with a high shear mixer (IKA Ultra Turrax T25, IKA, Germany) at 15000 rpm for 1 minute in a glass beaker.

The SC and SC.c formulations were prepared in a similar way, where the oil-phase and water-phase were mixed separately at 65-75 °C until all ingredients were dissolved, before adding the oil-phase to the water-phase while mixing with a propeller stirrer. The mixture was stirred for 5 minutes before emulsification with a high shear mixer at 12000 rpm for 1-2 minutes and allowed to cool down to room temperature during mixing.

Table 1. Composition of investigated cream formulations in (wt%).

Description	Functional category	Pickering cream	Surfactant-based replica of Pickering cream	Surfactant-based cream	Surfactant-based commercial cream
Cream id		PC.c	SC.c	SC	Canoderm® *
Water	Solvent	up to 100.0	up to 100.0	up to 100.0	Y
Glycerol	Humectants ^{1,2}	5.0	5.0	-	-
Urea		-	-	-	Y
Propylene glycol		-	-	-	Y
Glyceryl polymethacrylate	Film-forming ¹	-	-	-	Y
Carbomer	Rheology modifier	0.10	0.10	-	Y
Modified Quinoa starch	Stabilizing particles	10.0	-	-	-
PEG-100 stearate	Surfactants / emulsifiers ¹	-	2.0	2.0	Y
Glyceryl stearate		-	2.0	2.0	-
Cetearyl alcohol		-	2.0	2.0	Y
Polysorbate 60		-	-	-	Y
MCT-oil	Emollients	12.0	12.0	-	Y
Isotridecyl Isonnanoate		4.0	4.0	-	-
Canola oil		4.5	4.5	-	Y
Hydrogenated Coco-glycerides		3.0	-	-	-
Jjoba-oil		3.0	3.0	-	-
Solid paraffin		-	-	-	Y
Liquid paraffin oil		-	-	15.0	-
Dimethicone		-	-	15.0	Y
Tocopheryl acetate	Antioxidant ²	0.50	0.50	-	-
Phenoxyethanol/ Caprylyl glycol	Preservatives ¹	-	-	0.70	-
Ethyl paraben		-	-	-	Y
Propyl paraben		0.20	0.20	-	-
Methyl paraben		0.20	0.20	-	Y
Lactic acid	Buffering, water-binding ² /skin-conditioning ¹	-	-	-	Y
Citric acid	Buffering ¹	-	-	-	Y

*Composition according to ingredient list on product. Y = yes.

¹(Rowe et al., 2013)²(Lodén, 2005)

2.3 Preparation of skin substrates

Fresh porcine ears were acquired from a local abattoir and stored at -80°C . The ears were residuals from food preparation, and hence ethical permission is not required. To prepare the skin substrate, thawed pig ears were cleaned, and hair was removed with a trimmer. Full-thickness skin was excised from the inner ear using a scalpel and cut into strips ($2 \times 5 \text{ cm}^2$). Finally, the excised porcine skin was wrapped in aluminum foil and stored in -20°C until use. Before conducting tactile friction measurements, the skin was allowed to thaw and equilibrate at room temperature (at least 1 hour) with a hydrated filter paper underneath. Skin from different porcine ears and position on the ear was randomized among the measurements.

2.4 Sensory evaluation

Sensory perception evaluation was carried out on the four moisturizing cream formulations (PC.c, SC.c, SC and Canoderm®) for perceptual attributes during application and afterfeel. Thirty-one healthy untrained volunteers (19 females, 12 men), with no history of skin disease, were recruited among students and university personnel at the Faculty of Health and Society (Malmö University) to participate as assessors in the study in accordance with ISO 8586:2012. The age ranged between 18 and 64, whilst most participants were in the age range 25-34 (67.7%). Ethical approval was received by the Sweden Ethical Review Agency (DNR 2019-05452) to conduct the study, and informed consent was obtained by all volunteers.

The evaluations were performed with assessors seated in individual booths with partition walls and homogenous artificial lightning in a dedicated room isolated from external disturbances, with temperature and humidity control in accordance with ISO 8589:2007. The study was divided into sessions of 4-6 assessors per session. Prior to each session, the assessors were asked to wash and dry their hands and were introduced to the test procedure and the terminologies by the project leader. The introduction allowed for an automatic 20-30 minutes quiet acclimation period for the assessors. The assessors had been further asked to avoid caffeine beverages 3 hours before the session, not to cover their forearms, and not to apply any topical formulation 12 hours prior to the session in accordance with guidelines for *in vivo* measurements on skin (Berardesca et al., 2018; Otto et al., 2009; Pinnagoda et al., 1990).

Each sample was assigned a random three-digit code and presented in an identical dark glass container. The order of presentation was balanced by randomized complete block design to avoid first order and/or carry-over effects within sensory sessions. The sensory attributes were assessed on two defined circular measurement sites ($d = 4 \text{ cm}$) on each volar forearm, placed 7 cm from the wrist and elbow, and distanced 2 cm from each other. Each circle was labelled with a sticker with the formulation 3-digit code according to the randomized order. The assessors were given similar labels on the back of their fingers, as a reminder to use one finger for each measurement site to avoid carry-over effects and confusion.

In vivo baseline measurements of skin hydration for each measurement site were taken prior to application of 50 μl of each cream by the project leader to the middle of the circular measurement sites using a M1000 positive displacement pipette (Microman®, Gilson, France). Once the creams were applied, assessors were allowed to start the evaluation following the instructions on a digital survey (Microsoft Forms) on a tablet computer. The study was divided

between attributes evaluated during the application phase, and attributes evaluated in the afterfeel phase (11 minutes after application). The sensory attributes evaluated, and instructions are summarized in Table 2. After evaluating the attributes during application, the assessors were asked to clean their fingers with facial tissues and answer a questionnaire with background information during the waiting time before the afterfeel evaluation phase. The creams were ranked for each attribute according to the rank test (ISO 8586:2006) (Meilgaard et al., 2016; Whelan, 2017), rank ordering the four formulations from 1 to 4 for the highest to lowest perception for each specific attribute. After ranking the formulations, the assessors could choose their preferred cream formulation for each studied attribute. They were also asked to choose their overall preferred formulation during the application phase and between the creams overall. By the end of the study, the assessors were also asked to describe each formulation by choosing words from a word cloud, or by using their own words.

2.5 Skin hydration measurements

Skin hydration was measured *in vivo* during the sensory perception study before the application of creams, and upon completion of the study by electrical conductance by means of a hydration probe (Dermalab, Cortex Technology, Denmark) at circular measurement sites on the volar forearms. The instrument measures skin conductance at a single frequency, 300 kHz, which can be related to the water content of stratum corneum on an arbitrary scale (Morin et al., 2020). The results are given as the relative change (%) in μS (micro Siemens) on each measurement site to evaluate the effect of the formulations on skin hydration.

Ex vivo skin hydration measurements were performed during tactile friction measurements, using a Corneometer (CM825, Courage Khazaka Electronic GmbH) to measure the skin hydration of the finger and the excised skin, prior to each experiment. A second measurement was performed after each experiment on the excised skin. The results are given as the relative change (%) in arbitrary units (a.u.) on excised skin to evaluate the effect of skin hydration on friction results.

Table 2. The attributes evaluated using a ranking test, given in the order they were evaluated in the study with definitions and instructions as specified in the survey. 50 µl of each formulation was applied on circular sites on the volar forearms (two sites per forearm).

Attributes, <i>Application phase</i>	Definition	Instructions
Moistness	The amount of liquid (wetness) which can be perceived during application	Spread the sample droplet within the marked circle in a circular motion with 1 finger. (1 circle/second). After 3 circulations, evaluate how moist the samples are.
Spreadability	The ease to spread the sample over a larger skin area	Continue distributing the sample on the skin in circular motion (1 circle/second). After another 2 circulations (total 5), evaluate how spreadable the samples are.
Thickness	The amount of sample-film between the finger and the skin	Continue distributing the sample in a circular motion (1 circle/second). After another 7 circulations (total 12), evaluate the thickness of the samples.
Absorption	How fast the sample absorbs into your skin	Continue distributing the sample in a circular motion (1 circle/second). Max 18 additional circulations (total max 30) Evaluate how fast the sample is absorbed by your skin.
Attributes, <i>Afterfeel phase</i>	Definition	Instructions
Stickiness	How sticky/gluey the skin feels	Press your finger withing the marked circle and lift it to evaluate the stickiness. You may repeat it 3 times. Evaluate how Sticky the skin area feels.
Residual Coating	The amount of remaining product on the skin area	Use your finger to slide softly over the skin in the marked circles and evaluate how much product that remains.
Greasiness	Amount of oil/fat which can be perceived on the skin surface	Slide your finger within the marked circles and evaluate how greasy/oily the skin area feels.
Slipperiness	How slippery the skin feels	Let your finger slide over the skin in the marked circles and evaluate how slippery the area feels.
Softness	How soft and smooth the skin feels	Let the finger slide slowly in the marked circles and evaluate how soft the area feels.

2.6 Rheological studies

Rheological measurements were performed using a stress-controlled rheometer, Bohlin CVO100 (Malvern Instruments, UK) equipped with a parallel plate with a diameter of 25 mm and a gap size of 0.50 mm. The formulations were applied to the bottom plate with a stainless-steel spatula and analyzed at 32 °C. Continuous flow tests (hysteresis loop tests) of the formulations were carried out with shear rate ramp, by increasing the shear rate from 0.25 to 200 s⁻¹, hold time of 0.1 s, and decreasing to 0.25 s⁻¹. The duration of each step was 10 s. The steady shear viscosity and hysteresis loop area values were calculated from the obtained curves (shear stress vs shear rate) using the Bohlin Rheometer Software. Dynamic (oscillatory) tests were performed to determine the linear viscoelastic region (LVR) and yield stress of the samples, at constant frequency of 1 Hz and an amplitude sweep ramp from 0.24 to 1000 Pa. A frequency sweep was performed in the linear region from 0.1 to 1000 Hz at 0.3 Pa constant shear stress. The storage (G') and loss (G'') moduli phase angle (δ) was measured. The yield stress was determined from the onset value of the modulus curves in a double logarithmic plot of the storage modulus (G') as a function of oscillation stress (Dinkgreve et al., 2016; Walls et al., 2003).

2.7 Tactile friction measurements

Tactile friction measurements were performed using a ForceBoard™ (Industrial Dynamics Sweden AB, Järfälla, Sweden), following the method described by Skedung et al. (Skedung et al., 2016) with minor adjustments. A temperature-controlled plate was placed on top of the ForceBoard™ (32 °C) and excised porcine skin (2x5 cm²) was mounted on the heated plate with hydrated filter paper underneath. The friction force (F) and applied load (L) were continuously recorded as a finger interrogated the model skin surface by moving the index finger back and forth, and the friction coefficients (μ) were calculated as a ratio of the friction force and load according to:

$$\mu = \frac{F}{L} \quad (1)$$

Approximately 4-5 mg/cm² of the topical formulations (PC.c, SC, and Canoderm®) were applied to the skin substrate. For each experiment the index finger, inclined 30°, was stroked forward and back 10 times to spread 50 µl (20-25mg) of applied formulation over the sample area (4-5cm²). A control measurement on untreated skin substrate with 10 strokes was performed prior to each measurement as control for changes in skin and finger. The friction was measured for 30 seconds during application of the formulation at the time points 2.5-minutes, 5.5 minutes, and 11 minutes after application. All experiments were performed in controlled ambient conditions (temperature = 21 °C ± 0.3 °C, relative humidity = 49% ± 6%) by the same experimenter. The method was validated by comparison with another experimenter and an artificial skin substrate (Ali et al., 2021). The friction force and the applied load were recorded with a sampling rate of 100 Hz and the applied load was maintained around 0.5 N. The results are given as average friction coefficient values normalized against corresponding values for the untreated skin substrates to control for biological variation between individual skin substrates. The results for untreated skin substrates show a small reduction in normalized

friction coefficient over the measurement period of 11 minutes, tentatively resulting from water evaporation with time (Ali et al., 2021).

2.8 Statistical Analysis

Whenever applicable, results were expressed as averages $\pm$ standard deviation, and differences between cream formulations were determined by one-way ANOVA with Tukey's HSD test. Statistical outliers were excluded based on two-sided Grubbs' test ($p < 0.05$).

A Friedman test of difference was used to determine whether participants had a significant different rank ordered preference for the four cream formulations ($p < 0.05$). The Friedman test is the non-parametric alternative to the one-way ANOVA and shows whether significant differences exist between two or more samples that are ranked by all panelists. It is a very sensitive test to find a pattern of consistent rank order (Lawless and Heymann, 2010). Whenever there was significant difference, pairwise comparison was performed using Fisher's least-significant-difference (LSD) test to determine which of the cream formulations was significantly different ($p < 0.05$).

Multivariate analysis was carried out using Matlab (v. R2020b). Linear partial least squares regression analysis (PLS) was used to visualize the relationships between the studied creams, the rank sums of the sensory attributes and the mean data for the physical measurements.

3. Results

3.1 Participants' background

Questionnaire results provided background information about the participants in the sensory perception study (supplementary information). Most of the participants were females (61%), and a large number of the participants were in the age range 25-34 years old (67%). Almost half of the participants (48%) use hand cream on a daily basis, while the rest used it only on a weekly basis (19%), seasonally (19%), or never (4%).

Most of the participants preferred non-greasy (> 38%) and soft, cushioning (> 38%) moisturizing creams. Some participants (15%) preferred their moisturizing creams to be thin as well as non-greasy/soft, cushioning. Upon ranking the most important criteria when purchasing a new moisturizing cream, the top three criteria most important for the participants were the moisturizing effect, the afterfeel sensation, and texture. Based on this information we can conclude that the afterfeel sensation of the applied formulation is important for the participants in this study, and that a cream with a moisturizing effect and a non-greasy and soft afterfeel is more likely to be preferred.

3.2 Sensory perception evaluation

The results of the sensory study (Table 3) show statistically significant differences ($p < 0.05$) in participant's ranked preference between the four cream formulations for all sensory attributes except thickness and absorption. The rank sums of the cream formulations for each sensorial attribute are illustrated in a radar chart in Fig 1A.

Table 3. For each sensory attribute, Chi-square values (χ^2), and p-values from Friedman's test and rank sums for the cream samples with multiple comparisons using Fisher's least-significant-difference (LSD, $p < 0.05$). High rank sums correspond to high level of perception for each sensory attribute.

Attributes	χ^2	p-value	PC.c	SC.c	SC	Canoderm®
Moistness	9.62	0.022*	76 ^{ab}	81 ^b	92 ^b	61 ^a
Spreadability	19.26	<0.001*	66 ^a	92 ^b	94 ^b	58 ^a
Thickness	4.74	0.192	67 ^a	86 ^a	73 ^a	84 ^a
Absorption	6.60	0.086	73 ^a	66 ^a	91 ^a	80 ^a
Stickiness	32.26	<0.001*	44 ^a	78 ^b	94 ^b	94 ^b
Residual Coating	10.08	0.017*	62 ^a	94 ^b	75 ^{ab}	77 ^{ab}
Greasiness	16.59	<0.001*	55 ^a	96 ^b	79 ^b	80 ^b
Slipperiness	35.05	<0.001*	45 ^a	101 ^c	73 ^b	91 ^c
Softness	31.68	<0.001*	46 ^a	87 ^{bc}	76 ^b	101 ^c

* for significant results, $p < 0.05$

Creams sharing the same significance group letter (a,b c) show no difference in ranked perception.

Figure 1. Radar charts of rank sums (A) and preference score (B) of each cream formulation for the studied sensory attributes. Sensory attributes studied during the afterfeel phase are star marked. A: Rank sums of each cream formulation for the studied attributes. A higher value of rank sums for a certain sensorial attribute means a stronger perceived intensity for this attribute for a given formulation (*e.g.*, most soft). B: Preference score of each cream formulation for the studied attributes, showing which cream was most preferred for a specific attribute. The formulations were Pickering cream (PC.c) in green, surfactant-based cream replica of Pickering cream (SC.c) in blue, surfactant-based cream (SC) in yellow, and surfactant-based commercial cream (Canoderm®) in purple.

3.2.1 Perception during the application phase

The attributes evaluated during the application phase are relevant to the cosmetic appeal of creams, and formulations ranked high for these attributes are generally considered as cosmetically appealing for consumers. Moistness, and absorption are related to the sensation of the skin being moisturized and hydrated, while thickness and spreadability are related to a pleasant feeling and the ease of application. During the application of the topical creams to the skin, the formulations need to spread easily without feeling too greasy or sticky (Kwak et al., 2015). Formulations ranked high for these attributes can be considered as cosmetically appealing for consumers.

During the application phase, Canoderm® was perceived as less moist than SC.c and SC ($p < 0.05$), and together with PC.c it was perceived as less spreadable than SC.c and SC ($p < 0.05$). The highest rank sums overall for the sensory attributes during the application phase were given to SC for all sensory attributes except thickness, where SC.c and Canoderm® were higher. Thus, we can conclude that SC was perceived as the moistest, the easiest to spread, and the cream to absorb fastest by the skin during the application phase.

3.2.2 Perception during the afterfeel phase

According to the human panel in this study, the afterfeel sensation is an important criterion when purchasing a cream. Most participants preferred a non-greasy and/or soft afterfeel. Thus, a high rank sum value for softness and a low value of rank sums for greasiness can be interpreted as being cosmetically appealing for this specific panel. In general, some of the attributes related

to the afterfeel phase are commonly considered unfavorable; such as stickiness, greasiness and slipperiness (Kwak et al., 2015; Lodén M, 2003; Nacht et al., 1981), while softness can be seen as a favorable attribute, *e.g.*, a powdery afterfeel (Moussour et al., 2016; Timm et al., 2012). Low values of rank sums for stickiness, and slipperiness can also be considered as cosmetically appealing.

In the afterfeel phase, 11 minutes after the formulations were applied, the participants' ranked preferences showed that the lowest rank sums ($p < 0.05$) were given to PC.c for all afterfeel sensory attributes. The afterfeel of PC.c was thus perceived as the cream with least stickiness, greasiness, residual coating, slipperiness but also the least soft. Canoderm[®], SC and SC.c were perceived as sticky and greasy in afterfeel, with high rank sums for stickiness and greasiness. The highest amount of residual coating was perceived for SC.c in the afterfeel phase, significantly different from PC.c ($p < 0.05$). Furthermore, the residual films of SC.c and Canoderm[®] were perceived as most slippery on skin, significantly higher than PC.c and SC ($p < 0.05$) separately, and the cream that was perceived to leave the softest ($p < 0.05$) skin sensation in the end was Canoderm[®].

3.2.3 Preference choice

The assessors were also asked to choose the cream they preferred the most for each attribute during application and afterfeel phases, Fig. 1B. During the application phase, SC.c, was preferred the most for moistness and spreadability attributes while Canoderm[®] was the most preferred cream for thickness and absorption attributes. For the afterfeel attributes, Canoderm[®] was the most preferred for softness and slipperiness while SC was most preferred for stickiness, residual coating, and greasiness. SC was the overall most preferred cream for the application attributes. When asked for the most preferred cream overall, without thinking of a certain attribute, Canoderm[®] was the most preferred cream. Canoderm[®] is a fully developed product and marketed as an efficient moisturizer, in comparison to the prototypes prepared in the lab, and this difference was perceived by the assessors when they only needed to compare the overall performance between all four creams.

The preference results differed to some extent from the results by ranking. This highlights how the participants are in different state of mind when asked to compare and rank the creams based on level of perception for each attribute, and when asked to choose the cream they preferred the most. In the first case, they are in an analytical frame of mind when comparing and ranking, while they are looking at the cream as a whole and judging based on their own preferences when asked to choose the most preferred (Lawless and Heymann, 2010). Preference tests would require a larger number of assessors or trained panelists and preference results were only considered as supplement to the rank test.

3.2.4 Participants' descriptive words

At the end of the sensory study, the participants were asked to describe each cream formulation with their own words. A word cloud animation was provided as assistance. The words used to describe each cream formulation are illustrated in Fig. 2, with colors and font size highlighting how frequent each word was used. The words that were used the most to describe PC.c were

3.2.5 Hydration measurements *In vivo*

The results of the hydration measurement (Fig. 3) of the skin before and after the application of creams can help distinguish the degree of hydration to the skin after application of the creams. The relative change (%) in μS was significantly highest for Canoderm® ($p < 0.05$), followed by SC.c. Despite the fact that SC.c and PC.c have the same composition of excipients and differ only by the addition of starch particles instead of surfactants, the relative change in skin hydration was significantly higher for SC.c ($p < 0.05$). The fatty and hydrophobic composition of the surfactants can contribute to an occluding effect giving rise to a higher value in skin hydration, while the presence of starch particles may alter the occluding properties of the residual oil film.

The difference between the surfactant-based creams could be related to difference in oil composition and presence of humectants. Furthermore, Canoderm® is marketed as an efficient moisturizer with 5% urea as well as propylene glycol as humectants, compared to glycerol which is present in PC.c and SC.c. Urea has been shown to be an efficient humectant to increase skin hydration (Albèr et al., 2013), resulting in lower TEWL in comparison to glycerol (Lodén et al., 2001). Hence, addition and choice of humectants together with occlusive emollients significantly affect the moisturizing properties of a cream.

Figure 3. Relative change in conductance, μS , after application of cream formulations *in vivo*. Results are expressed as average ($n = 30$) and error presented as standard deviation, and differences between cream formulations were determined by one-way ANOVA with Tukey's HSD test. Creams with different group letter (a, b, c, d) are significantly different ($p < 0.05$) in relative change in hydration.

3.3 Rheological studies

3.3.1 Flow curves

The flow properties of a cream can tell us how easy it is to apply and spread a cream on the skin. It has been suggested that a skin cream should have low viscosity at high shear to be easy to apply, and high viscosity at low shear so it does not spill out of the container (Greenaway, 2010). The flow behavior of the studied creams (Fig. 4) show that all creams exhibited shear-thinning and thixotropic behavior. It can be noted that steady shear viscosity (at low shear rate) was highest for Canoderm[®] and PC.c, followed by SC.c and SC. The creams with higher viscosity all had in common the presence of a structural thickener (carbomer). The presence of starch in the PC.c could explain the higher initial viscosity of PC.c in comparison to SC.c. SC exhibited the lowest shear viscosity and differed the most from the other creams.

Figure 4. Shear-thinning behaviour for all formulations. Pickering cream (PC.c), green circles, surfactant-based Pickering cream replica (SC.c), blue triangles, surfactant-based cream (SC), yellow diamonds, and surfactant-based commercial cream (Canoderm[®]), purple squares.

3.3.2 Oscillation measurements

Yield stress is a measure of the stress required to induce flow in a product and can be related to the interparticle structure. Results from oscillation amplitude sweep measurements are shown in Table 4.

All formulations showed elastic behavior at low stress, indicated by higher G' values than G'' values, and displayed a linear viscoelastic region (LVR) up to approximately 3 Pa. Upon further increase of the shear stress, the G' values drop for some formulations and the viscous flow effects is apparent with G'' leading to cross over. Yield stress results were determined from the onset of the curve of storage modulus (G') (Dinkgreve et al., 2016) (Figure S1, Supplementary

information) and summarized in Table 3. The highest yield stress recorded was 129.7 Pa for Canoderm®. PC.c exhibited lower yield stress (77.8 Pa) than Canoderm® but higher than SC.c (33.2 Pa). The difference between PC.c and SC.c can be attributed to the difference in using surfactants or starch particles as emulsion stabilizers and the difference in microstructure. The lower yield stress of SC.c indicates increased breakdown of the structure in comparison to PC.c. The only cream which did not include any structural thickener was SC, and it exhibited the lowest yield stress values (< 16.8 Pa).

Carbopol as a structural thickener is known to enhance stability, and add body to skin cream samples (Epstein, 2009; Greenaway, 2010; Kwak et al., 2015). Therefore, it is not surprising that formulations including carbopol displayed higher yield stress and viscosity. However, differences beyond the addition of carbopol could relate to the amount or type of oil used and the microstructure of the oil droplets. According to Brummer et al. (Brummer and Godersky, 1999) the onset of flow for creams is above a critical shear stress of 10 Pa, whereas lotions begin to flow at lower yield stresses <10 Pa. The four main formulations investigated here all displayed yield stress values above 10 Pa indicating that they are suitable as topical creams.

Table 4. Rheological parameters of investigated formulations. G' , G'' , and phase angle values were obtained at 1 Hz. Yield stress was obtained from the onset of the curve of G' . Steady state viscosities and hysteresis loop areas were obtained from flow tests. Values are given as mean $\pm$ SD.

Creams	Temp. (°C)	Hysteresis	Steady shear		G'' (Pa)	Tan δ	Yield Stress (Pa)
		Loop area (Pa s ⁻¹)	viscosity (Pa s)	G' (Pa)			
Canoderm®	32	4913 $\pm$ 423	460.8 $\pm$ 68.6	6181 $\pm$ 1276	3287 $\pm$ 1534	0.51 $\pm$ 0.1	129.7
PC.c	32	2846 $\pm$ 417	288.7 $\pm$ 50.3	871 $\pm$ 178	69.2 $\pm$ 7.5	0.08 $\pm$ 0.02	77.8
SC	32	1875 $\pm$ 15	28.5 $\pm$ 5.9	619 $\pm$ 201	229 $\pm$ 71.1	0.37 $\pm$ 0.01	16.8
SC.c	32	3171 $\pm$ 37	105.4 $\pm$ 20.3	1277 $\pm$ 115	359 $\pm$ 86	0.28 $\pm$ 0.04	33.2

A frequency sweep was performed for all formulation at a value of shear stress within the LVR at 0.3 Pa (Table 4). The values of G' were higher than G'' ($\tan \delta < 1$) for all the formulations in a wide frequency range (up to 10 Hz), indicating elastic behavior and that all samples are in a gel (or solid) state. Above 10-29 Hz, the G'' crosses over and becomes higher than G' ($\tan \delta > 1$) showing that viscous behavior starts to dominate, and the samples enter into a liquid state. The values of $\tan \delta$ (G'/G'') were lowest for PC.c, which suggest that it is the most elastic sample with sturdier internal structure.

3.4 Tactile Friction

During initial application of a cream, when the film formed between skin and finger is rather thick, the friction is highly affected by the viscosity of the formulation, and the friction properties are related to the perceived slipperiness of the product (Skedung et al., 2016; Tang et al., 2015). After spreading of the formulation, as the water and other volatile components evaporate, the effect of the product on the skin can be detected. For example, hydrated and soft

skin gives rise to a higher friction, while a large amount of non-adsorbed oil residue on the skin may give rise to lower friction (Nacht et al., 1981; Skedung et al., 2016; Tang et al., 2015).

The average normalized friction coefficients during application on skin, and 2.5, 5.5 and 11 minutes after application of studied cream formulations are shown in Fig. 5. Upon application (0 min), there is an initial drop in friction of the skin due to spreading for all the creams in comparison to the untreated skin samples. Once the thickness of the formulation film is decreased and subsequent changes of the residual film occur, including the evaporation of volatile compounds, the normalized friction is increased for all formulations. PC.c shows a pronounced increase in friction after 2.5 minutes, and by the end of the experiment (11 min), the friction of PC.c is still higher than untreated skin. Canoderm[®], SC.c and SC displayed a minor gradual increase in friction over the measuring time. After 11 minutes, Canoderm[®] reaches similar friction levels as untreated skin, while the friction for SC.c and SC (SC.c > SC) is lower than that of untreated skin. The difference in friction between PC.c, SC.c, SC, and Canoderm[®] can be related to the use of starch particles instead of surfactants. The residual film in Canoderm[®], SC.c and SC contains oils and surfactant residues which could contribute to more slippery and greasy tactile properties reducing the friction. As for PC.c, the residual film contains mainly oils and starch particles, where the presence of starch particles may contribute to higher tactile friction and counteract the reduction in friction caused by the residual oil. Nacht et al. (Nacht et al., 1981) concluded that greasy products have been found to induce an initial decrease in friction coefficient, while products that increase friction coefficients would be perceived as non-greasy. Thus, high friction inducing PC.c can be considered as non-greasy, while surfactant-based creams with lower friction can be perceived as greasy. Timm *et al.* showed that adding particles to an aqueous suspension lowered friction and friction lowering was particle size dependent (Timm et al., 2012). However, no comparisons were made with emollients or particle containing creams. It is thus not farfetched to assume that, once water evaporates from the formulation, the friction would differ between a dry particle residue after an aqueous suspension, and a residue of particles and oil mixed after an emollient suspension or a cream containing particles. Furthermore, the microstructure of the residual film may also differ for particles dispersed in a surfactant-based cream, or particles used as emulsifiers surrounding the oil droplets (Pickering emulsions). Difference between SC.c, SC and Canoderm[®] may be attributed to the different properties of the residual film due to different emollient and surfactant composition.

Figure 5. Tactile friction results on excised skin at 32 °C of the studied formulations. Surfactant-based cream (SC) in yellow, surfactant-based commercial cream Canoderm® in purple, surfactant-based cream with carbomer (SC.c) in blue, and Pickering cream (PC.c) in green. One-way ANOVA showed that the SC and PC difference at 2.5 min and 5.5 min is statistically significant ($p < 0.10$).

3.5 Hydration measurements *Ex vivo*

The relative change (%) in skin hydration (a.u) of excised skin before and after the experiment for the different treatments is shown in Fig. 6. For untreated skin samples, a reduction in skin hydration (up to 27%) was noted for the experiment period of 11 minutes. Thus, the water loss from the hydrated excised skin due to evaporation was high. There was tendency for relative increase in hydration 11 min after application observed for all creams except SC, but no significant differences in hydration were found among the creams and untreated skin. The increase in hydration was highest for Canoderm® followed by PC.c and SC.c. Canoderm® comprises humectants such as urea and propylene glycol which may have contributed to the higher skin hydration in combination with occlusive effects of the emollient composition. PC.c and SC.c both comprised glycerol as humectant, and although starch particles in PC.c may alter the properties of the occlusive film, the skin hydration for PC.c was still higher than SC.c. SC did not include any humectants and contained different emollient composition which may have contributed to the lower skin hydration. Thus, humectants when combined with an appropriate emollient composition had a larger impact on skin hydration results. The higher skin hydration levels of Canoderm® may explain the difference in friction results between Canoderm®, SC.c and SC, since hydrated skin increases friction. Canoderm® was also the cream that increased the participants skin hydration the most *in vivo*.

Figure 6. Change in a.u. % after application of formulations on excised skin (11min). Surfactant-based cream (SC), yellow bar, commercial cream Canoderm®, purple bar, surfactant-based cream with carbomer (SC.c), blue bar, Pickering cream (PC.c), green bar, and untreated skin substrates, textured bar. Errors are presented as standard deviations.

4. Discussion

4.1 Relationship between sensory and physical data and creams

In this study we have conducted an extensive study of both sensory properties and physical chemical properties of four creams. Sensory properties are always the key attribute from a consumer perspective, but consumer panels are time consuming and thus not a fast tool during the development phase. Consequently, there is a need to link the sensory properties to easy to measure physical properties.

We conducted partial least square (PLS) mainly as a visual tool (due to limited numbers of studied creams) to study the relationships between physical data, sensory data, and the studied creams. Sensory data comprised rank sums of the creams for each sensorial attribute evaluated by thirty-one assessors, and the physical data comprised average data of replicates ($n=3-4$) for each cream and physical parameter. PLS showed that the main data variance, 62.46 %, was explained by the first two principal components. The first principal component (PC1) explained 38.07% of the data variance , while the second principal component (PC2) accounted for 24.39% of the variance. A PLS plot combining the sensory data, physical data, and the studied creams (Fig. 7.) suggests that PC1 is associated with afterfeel attributes and physical data, since they are generally distributed along the x-axis. Tactile friction data and *ex vivo* hydration data are positioned on the positive side of the x-axis and rheological and *in vivo* hydration data are, in general, on the negative side of the x-axis. PC2 seems to be associated with sensory attributes related to the application phase, due to their apparent distribution along the y-axis. In general, it seems as if PC2 shows differences between moist and thin creams and fatty and thick creams.

The four studied creams were distributed quite well in both dimensions of the PLS, indicating that each cream appears to have unique characteristics in their perceived attributes. PC.c is negatively associated with most afterfeel attributes (greasiness, residual coating, and stickiness) suggesting it was perceived to have less residual coating and to be less greasy and sticky than the other creams. It was also the cream that is positively associated with tactile friction data. Canoderm[®], appears to be positively associated with thickness, and negatively associated with moistness. Furthermore, Canoderm[®] seems to be positively associated with rheological data and hydration data and differed most from the other three creams in these aspects. SC is influenced slightly more by application attributes than afterfeel attributes and is associated with spreadability since it was ranked high for spreadability and perceived as easy to spread during application. SC is however negatively associated with friction data, indicating that it has properties that result in low friction values. The PLS suggests that SC.c had rather weak associations with the various variables, suggesting ‘middle-of-the-road’ physical measures and sensorial responses.

The surfactant-based creams appear to be separated from each other along the y-axis suggesting slightly different characteristics between the creams and mainly associated with hydration and rheological results. For instance, differences between a moist and thin cream and a thick and fatty cream can be visualized along the y-axis. On the other hand, the relationship between surfactant-based and surfactant-free creams was mainly explained by the positioning along the x-axis. Furthermore, strong differences in afterfeel perception between the surfactant-based creams due to type of emollient used could to some extent be visualized by their position along the x-axis. Thus, the differences in composition due to oil:water ratio, type of emollient used, and presence of thickeners and humectants could mainly be explained by their position along the y-axis, and partly by their position on the x-axis, and can be studied by means of hydration measurements and rheological studies.

Figure 7. Partial least square (PLS) visualizing the relationships between sensory data (circles), physical data (black stars) and studied creams. Sensory data are shown as attributes studied during the application phases (white circles) and during the afterfeel phase (black circles). The studied creams are PC.c (green circle), SC (yellow diamond), SC.c (blue triangle), and Canoderm® (purple square).

4.2 Relationship between sensory and physical data

Tactile friction results and afterfeel attributes are generally negatively associated with each other, while rheological and hydration data seems to be related to some afterfeel attributes. The PLS further suggests that there seems to be negative associations between absorption and tactile friction data in general. Spreadability seems to be negatively associated with friction results at 2.5 minutes, suggesting that a cream with a residual film that remains spreadable after 2.5 minutes is likely to have low friction values. Furthermore, spreadability seems to be negatively associated with the yield stress, as a cream with a high yield stress value is more likely to be difficult to spread. Negative associations were also found for moistness and the storage modulus (G'), the viscous modulus (G''), viscosity, and *in vivo* hydration data. Moistness was defined as the amount of liquid or degree of wetness perceived during application. Thus, it is logical to imagine that a thin cream with low viscosity is perceived as more moist by the participants. However, skin hydration measurements focus on evaluating water content of the stratum corneum by means of capacitance and conductance, and are not appropriate methods to measure moistness on skin (Berardesca et al., 2018). Thus, we cannot suspect any logical causality between moistness and hydration results.

The PLS further shows that thickness and softness were positively associated with each other and associated with viscosity results.

The present results, as visualized by PLS, suggest that tactile friction experiments could be used to determine sensorial attributes related to afterfeel, and rheological experiments could be used to determine attributes relating to thickness, and spreadability.

4.3 Impact of excipients on sensorial and physical properties

The results show that the functional excipients used had an impact on physical properties that could be related to sensorial properties. Humectants were the only exception since the impact of which could only be measured instrumentally, and the moisturizing effect was not evaluated by perception during the evaluation period. Moreover, although humectants can give a moisturizing effect that can change the aesthetics of the skin after long-term use, it is unlikely that this change can be perceived short after one single application.

The presence of a rheological thickener contributed to the viscosity, yield stress and the storage stability of creams. Consequently, the viscosity and yield stress were mainly related to the initial application and spreading of the creams. The two creams with lower viscosity and yield stress, SC and SC.c, were perceived as easiest to spread. However, SC did not contain any thickener and differed from SC.c in the emollient composition. Furthermore, SC was also perceived as the moistest and quickest cream to be absorbed during application. Thus, the combination of thickener and emollients is important to consider when designing a formulation with specific physical properties that can be perceived as cosmetically appealing during the application phase.

Humectants, in combination with occlusive emollients, proved to be very important for sufficient moisturizing effects. Furthermore, the increased skin hydration results after application of Canoderm® highlights the difference in hydration efficiency between urea and glycerol. The perception study did not emphasize any effect in sensorial properties that could be related to the moisturizing effect. However, small differences regarding tactile friction between Canoderm® and SC, could perhaps be related to increased skin hydration for skin samples treated with Canoderm®. This observation is in agreement with similar observations made by Lodén (Lodén et al., 1992) and Skedung (Skedung et al., 2016), however in neither case was skin hydration measured.

The choice of the right emollient composition is important for the sensorial properties of a cream. The results showed that the different emollient composition may be the explanation for the difference seen between the surfactant-based creams. Canoderm® and SC.c were ranked highest for softness, and both creams contained dimethicone which is known for its sensory benefits. However, due to the variety of emollients used in the emollient mixture it was not possible to conclude which type of emollient that had a greater influence on the physical and sensorial properties. The amount of emollient used in the prototype creams (PC.c, SC, SC.c) was however almost similar (28-30 wt%) while Canoderm® can be assumed to have lower emollient concentration with different surfactant composition (patent SE511551.C2).

The use of starch particles replacing traditional surfactants as emulsion stabilizers in a cream resulted in large differences in the perceived sensory properties, as well in the physical properties of the cream. These differences resulted in lower rank preference for sensory attributes important during the application phase of creams. Nonetheless, the overall sensorial benefit of the addition and use of starch particles as emulsion stabilizers could be related to the afterfeel sensation of creams. Generally unpleasant afterfeel attributes such as perception of a residual coating, stickiness and greasiness can be reduced and replaced by the sensation of a dry, powdery afterfeel.

Interestingly, PC.c was not perceived as a thick cream during the application, although the rheological results showed higher viscosity and yield stress values than for SC.c. Furthermore, PC.c was not considered soft and smooth, and one explanation could be that the assessor's connected soft and smooth to the thickness and body of the cream, rather than the soft afterfeel sensation as a result of presence of particles. Instead, Canoderm® and SC.c were considered thick and soft, and this could be connected to presence of fatty alcohols among the surfactants, contributing to the body and thickness of the creams that can be perceived as soft, cushioning, and smooth.

We can conclude that the right combination of a thickener, humectant, emollient composition, and emulsifier composition is needed in order to create a cream that can fulfill the stability and packing demands, and at the same time fulfill the sensory and moisturizing requirements. The use of starch particles to create a Pickering cream shows great possibilities to design surfactant-free creams that can meet the demands for sensitive skin and sustainable skin care products with unique sensorial properties.

For instance, to fulfill the requirements of the participants in the sensory panel in this particular study, a formulator would need to design a cream that is soft upon application, and non-greasy in afterfeel, and with a good moisturizing effect. With the knowledge in hand, a combination of the moisturizing property and softness of Canoderm® with the non-greasy, non-sticky, powdery afterfeel of PC.c would be needed to fulfill consumer demands.

5. Conclusions

The results show that tactile friction measurements on excised skin can be used to predict sensorial attributes related to the afterfeel sensation. Attributes related to the application phase such as thickness and spreadability can be predicted by means of rheological measurements.

The type of emollients used, as well as the presence of thickeners had the greatest impact on the difference in rheological and sensory results among surfactant-based creams. Differences between a starch-based Pickering cream and surfactant-based creams was more evident in relation to the afterfeel attributes, and easily distinguished using tactile friction measurements.

A starch-based, surfactant-free Pickering cream with higher friction coefficient values ranked low for greasiness, stickiness, slipperiness, and softness. Among surfactant-based creams, the difference in sensorial and physical properties between Canoderm®, SC.c and SC could be attributed to content of humectants and thickeners, the emollient composition, and surfactant composition.

6. Acknowledgements

We are grateful to the Knowledge foundation (Sweden) for funding the project, and Johan Engblom also thank the Gustaf Th Ohlsson foundation (Sweden) for financial support. Speximo AB is acknowledged for providing starch particles.

7. Conflict of interest

The authors declare no conflict of interest.

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Supplementary Information

Background information

Questionnaire

1. Skin Type

Please specify what skin type you think you have to the best of your knowledge

Normal Oily Dry Sensitive

2. How often do you use moisturizing cream?

Seasonal Weekly Never Daily

3. What type of skin cream do you prefer?

Thick, oily Thin Soft, Cushioning Non-greasy

4. Please rank the most important criterion for you when choosing a cream

Price, Moisturizing effect, Texture, Afterfeel, Sustainable, Eco-friendly, Brand, Ingredients

Yield stress measurement

Figure S1. Storage modulus as a function of shear stress. Yield stress measurement. Pickering cream (PC.c) in green circle, surfactant-based Pickering cream replica (SC.c) in blue triangle, surfactant-based cream (SC) in yellow diamond, surfactant-based commercial cream (Canoderm®) in purple squares.

III

Tactile friction of topical creams and emulsions: Friction measurements on excised skin and VitroSkin[®] using ForceBoard[®]

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Keywords: tactile friction, topical creams, VitroSkin[®], Force-Board[®], Pickering emulsion, excised skin, human panel

Abstract

Tactile perception can be investigated through *ex vivo* friction measurements using a so-called ForceBoard™, providing objective assessments and savings in time and money, compared to a subjective human panel.

In this work we aim to compare excised skin versus VitroSkin® as model substrates for tactile friction measurements. A further aim is to detect possible differences between traditional surfactant-based creams, and a starch-based Pickering cream and investigate how the different substrates affect the results obtained.

It was found that the difference in tactile friction between excised skin and VitroSkin® was small on untreated substrates. When topical creams were applied, the same trends were observed for both substrates, although the frictional variation over time relates to the difference in surface structure between the two substrates.

The results also confirmed that there is a difference between Pickering based formulations and surfactant-based creams after application, indicating that the latter is greasier than Pickering cream. It was also shown that the tactile friction of Pickering emulsions was consistently high even with high amounts of oil, indicating a non-greasy, and non-sticky formulation. The characteristics of starch-stabilized Pickering formulations make them promising candidates in the development of surfactant-free topical formulations with unique tactile properties.

1. Introduction

Tactile perception such as greasiness and stickiness are examples of key attributes for topical skin creams. Tactile perception is a complex combination of sensory stimuli including skin stretching, pressure applied, and vibrations during touching (Ding and Bhushan, 2016), and is an elusive sensation to capture. However, several authors have noted a correlation between sensorial evaluation using trained and untrained panellists and different methods to measure skin friction (Ali et al., 2021; Egawa et al., 2002; Savary et al., 2019; Tang et al., 2015), *e.g.*, the parameter greasiness has been seen to be inversely proportional to the friction (Ali et al., 2021; Nacht et al., 1981). Lately it has been shown that tactile friction, quantified with a ForceBoard™ (Industrial Dynamics Sweden AB, Järfälla, Sweden) is often a useful descriptor of perceptual experiences, and Skedung et al. has introduced a new term - psychotribology - that merges the fields of biotribology and tactile perception (Skedung et al., 2018). Although a physical method such as tactile friction cannot describe all types of perceptions that a human panel will identify, it has some obvious advantages such as providing objective assessments, and avoiding the inherent spread in results shown by a panel. It is also easier and cheaper to perform, and it focuses on one or a few parameters.

The friction of skin has been seen to be affected by humidity (Arvidsson et al., 2017; Derler and Gerhardt, 2012; Skedung et al., 2016), time of application and type of substrate used (Ding and Bhushan, 2016). Furthermore, it is also affected by the composition of the topical product such as presence/absence of occlusive components (Skedung et al., 2016), added moisturizers (Lodén et al., 1992), oil content (Nacht et al., 1981), and particles present (Ali et al., 2021; Timm et al., 2012).

The process of lubrication when stroking a finger over time on a body site or surface with a fluid layer has been divided into three main lubrication regimes, *i.e.*, the “boundary”, “mixed”, and “hydrodynamic” regime (Guest et al., 2013). The appearance of these lubrication regimes is thought to depend on the characteristics of the applied cream and the formed film thickness. Hydrodynamic lubrication occurs during the application of the cream when the film between the finger and the substrate is thick, and friction is mainly influenced by the formulation viscosity and thickness. Subsequent spreading of the formulation and evaporation of volatile compounds leads to a residual thin film. At this stage, mixed lubrication may occur leading to a moderate increase in friction as result of the changing properties of the residue and/or the underlying surface. It has indeed been shown that hydration of the surface by an aqueous vehicle or by occluding effects can increase friction over time (Nacht et al., 1981; Tang et al., 2015). When the thickness of the residual film is reduced, lubrication resembles the boundary regime and the friction will be dominated by the surface properties of the substrate (Guest et al., 2013; Tang et al., 2015).

Lately we have observed large differences between a Pickering cream and a traditional cream (Canoderm®) when it comes to perception of tactile properties as well as tactile friction (Ali et al., 2021). Pickering creams are particle-stabilized creams and they have attracted quite some interest for providing a possibility to produce surfactant-free creams with long shelf life (Frelichowska et al., 2009b, 2009a; Marku et al., 2012; Rayner et al., 2014; Wahlgren et al., 2013).

In skin research one of the challenges is to find suitable substrates for *in vitro* investigations. Traditionally, excised porcine skin (henceforth called “excised skin”) is one of the more common substrates and considered to be a good substitute for human skin (Haigh and Smith, 1994). During the last decade, several different *in vitro* skin models have been developed (Flaten et al., 2015; Van Gele et al., 2011). One driving force has been the need to avoid animal testing of cosmetic products. These models range from complex systems, like so called living skin equivalents, to fully synthetic substrates having *e.g.*, similar topography and/or lipid content as human skin. One of the synthetic models is VitroSkin® that has been successfully

used in investigations of sunscreen creams (Oliveira et al., 2008) and tactile friction studies of skin creams (Skedung et al., 2016; Tang et al., 2015).

In this work we aim to compare VitroSkin[®] and excised skin as substrates for tactile friction measurements. A further aim is to detect possible differences between a traditional surfactant-based commercial cream (Canoderm[®]), and a Pickering cream and investigate how the different substrates affect the results obtained. In addition, we have performed a small study on the effect of changing the composition using Pickering emulsions. Possible effects on the results by employing two different operators were also investigated.

2. Material & Methods

2.1 Materials

Canoderm[®] cream (ACO Hud Nordic AB, Stockholm, Sweden) was purchased from a local pharmacy. Modified quinoa starch, used as stabilizing particles in the Pickering formulations, was kindly provided by Speximo AB (Lund, Sweden). The starch was hydrophobically modified with octenyl succinic anhydride (Timgren et al., 2013).

The emollients used, medium chain triglycerides (Miglyol 812N, IOI Oleochemical, Hamburg, Germany), isotridecyl isononanoate (Crodamol TN, Croda, East Yorkshire, England), canola oil (Akosoft 36 and Lipex Preact, AAK, Karlshamn, Sweden), jojoba oil (Natura-Tec, Fréjus, France) and triolein (Captex GTO, Abitec Co, Janesville, USA), were purchased from Barentz (Copenhagen, Denmark). Other functional excipients were carbomer (Carbopol Ultrez 30, Lubrizol, Brussels, Belgium) used as a rheological modifier, and tocopheryl acetate (Dermofeel E74, Evonik Dr Straetmans, Hamburg, Germany) used as an antioxidant. Glycerol (Sigma-Aldrich, Stockholm, Sweden) was used as a humectant. The preservatives, propyl-4-hydroxybenzoate (Propyl paraben, Solbrol P) and methyl-4-hydroxybenzoate (Methyl paraben, Solbrol M), were provided by Lanxess GmbH (Leverkusen, Germany). Milli-Q water, 18.2 MΩ cm resistivity, was used for all samples. The surfactants used were cetearyl alcohol (Nafol 1618H, Sasol Performance Chemicals, Hamburg, Germany), and PEG-100 stearate (Myrj S100) and glyceryl stearate (Cithrol GMS 40) obtained from Croda Europe (East Yorkshire, England).

2.2 Formulations

The cream formulations studied in this work include a traditional surfactant-based commercial cream (Canoderm[®]), a surfactant-free, particle-stabilized Pickering cream, and a surfactant-based Pickering replica (referred to as Surfactant cream). All the formulations were oil-in-water (o/w) emulsions. The Pickering cream was prepared by mixing the water-soluble and oil-soluble excipients separately. The oil phase was heated to 60 °C until all emollients were melted, and the temperature was then brought down to < 45 °C. The starch was added to the water phase below 45 °C with stirring, propeller stirrer from IKA, Germany, and mixed for additional 5-10 minutes before slowly adding the oil-phase with stirring. The mixture was mixed for an additional 5-10 minutes before emulsification with a high shear mixer (IKA Ultra Turrax, Germany) at 15000 rpm for 1 minute in a glass beaker.

Canoderm[®] was used as is without any modifications. The compositions of the three creams are shown in Table 1. The oil content of Pickering cream was 28 wt%, while the oil content in Canoderm[®] could be assumed to be approximately 16 wt% with a total of 8 wt% emulsifiers in the oily phase based on a recipe in a corresponding patent (SE511551C2).

The Surfactant cream was prepared in a similar way, where the oil-phase and water-phase were blended separately at 65-75 °C until all ingredients were dissolved, before adding the oil phase to the water phase while stirring with a propeller stirrer. The mixture was stirred for 5 minutes

before emulsification with a high shear mixer at 12000 rpm for 1-2 minutes and allowed to cool down afterwards at room temperature.

Pickering emulsions were prepared at room temperature, according to the composition in Table 2, by mixing starch and water for 5-10 minutes and adding the oil with stirring and continue mixing for additional 5-10 minutes before emulsification with a high shear mixer (IKA Ultra Turrax, Germany) at 22000 rpm for 1 minute.

Table 1. Composition of creams.

Topical Cream	Ingredient list (INCI)
Pickering cream	Aqua, quinoa starch particles, caprylic/capric triglyceride, glycerol, canola oil, isotridecyl isononaoate, jojoba oil, hydrogenated coco-glycerides, carbomer, tocopheryl acetate, methylparaben, propylparaben.
Canoderm®*	Aqua, caprylic/capric triglyceride, urea, propylene glycol, hydrogenated canola oil, cetearyl alcohol, glyceryl polymethacrylate, dimethicone, paraffin, sodium lactate, carbomer, glyceryl stearate, PEG-100 stearate, polysorbate 60, lactic acid, citric acid, methylparaben, propylparaben.
Surfactant cream)	Aqua, caprylic/capric triglyceride, glycerol, canola oil, isotridecyl isononaoate, jojoba oil, hydrogenated coco-glycerides, PEG-100 stearate, glyceryl stearate, cetearyl alcohol, carbomer, tocopheryl acetate, methylparaben, propylparaben.

* Composition according to ingredient list on product, and order of ingredients according to (Lodén et al., 1999)

Table 2. Composition of investigated Pickering emulsions containing triolein in (wt%).

Ingredients	Pickering emulsion 5 wt% oil	Pickering emulsion 10 wt% oil	Pickering emulsion 18 wt% oil	Pickering emulsion 26 wt% oil
Water, up to	100.0	100.0	100.0	100.0
Modified Quinoa starch	1.6	3.3	6.6	10.0
Triolein	4.8	9.8	19.8	29.8

2.3 Preparation of substrates

2.3.1 Preparation of excised porcine skin

Fresh porcine ears were acquired from a local abattoir and stored at -80°C . The ears are residuals from food preparation, and hence ethical permission is not required. To prepare the skin substrates, defrosted porcine ears were cleaned and hair was removed with a trimmer. Full-thickness skin was excised from the inner ear using a scalpel and cut into strips ($2 \times 5 \text{ cm}^2$). Finally, the excised skin was wrapped in aluminium foil and stored at -20°C until use. Before conducting tactile friction measurements, the skin was allowed to thaw and equilibrate at room temperature (at least 1 hour) with a hydrated filter paper underneath.

For scanning electron microscopy, small pieces of $500 \mu\text{m}$ thick porcine skin were obtained using a dermatome (Dermatome, Integra LifeSciences, Plainsboro, NJ, USA).

2.3.2 Preparation of VitroSkin®

Vitro-Skin® (IMS Inc., Portland, ME, USA) was used as a synthetic model skin substrate. It is designed to mimic the human skin surface properties in terms of topography, pH, elasticity, surface tension, and ionic strength (IMS Inc.). The substrate (thickness $\sim 127 \mu\text{m}$) was cut into pieces of $2 \times 5 \text{ cm}^2$ and placed in a desiccator above a beaker with a mixture of 85 wt% Milli-Q water and 15 wt% glycerol for 16-24 hours before use according to the manufacturer's

instructions. This allowed for reproducible hydration of the substrate prior to friction measurements.

2.4 Tactile friction measurements

Tactile friction measurements were performed using a ForceBoard™ (Industrial Dynamics Sweden AB, Järfälla, Sweden), following the method described by Skedung et al. (Skedung et al., 2016) with minor adjustments. The friction force (F) and applied load (L) were continuously recorded as a finger interrogated the excised skin or the VitroSkin® surface by moving the index finger back and forth, and the friction coefficients (μ) were calculated as a ratio of the friction force and load according to:

$$\mu = \frac{F}{L} \quad (1)$$

A temperature-controlled plate was placed on top of the ForceBoard™ and the substrates were mounted on the heated plate (32°C). Excised skin (2x5 cm²) was mounted with hydrated filter paper underneath and pinned to the surface whereas pre-hydrated VitroSkin® (2x5 cm²) was attached with double-adhesive tape. Before each experiment, a measurement with 10 strokes on untreated substrate was performed as control for changes in skin and finger. For each experiment the index finger, inclined 30°, was stroked forward and back 10 times to spread 4-5 mg/cm² of formulation (*i.e.*, Canoderm®, Pickering cream, Surfactant cream and Pickering emulsions) on the substrate. The friction was measured for 30 seconds during application of the formulation and, 2.5, 5.5, and 11 minutes after. All experiments were performed in triplicates and in controlled ambient conditions (temperature = 21.5 ± 1.0 °C, relative humidity = 43.7 ± 10.3 %). The friction force and the applied load were recorded with a sampling rate of 100 Hz and the applied load was maintained around 0.5 N. The normalized friction coefficient was calculated by dividing the measured friction force for the topical formulations with the control measurement on untreated skin substrate. This means that a sample with a greater friction coefficient than 1 displays higher tactile friction than the untreated substrate.

A Corneometer (CM825, Courage Khazaka Electronic GmbH) was used to measure the skin hydration of the finger and the excised skin, prior to each experiment. A second measurement was performed on the excised skin after each experiment. The measured skin hydration is given in arbitrary units (a.u). For experiments done on VitroSkin® only the hydration of the finger was measured prior to and after each experiment.

2.5 Scanning Electron Microscopy

Samples were prepared by applying creams on excised skin and VitroSkin® separately by stroking the cream with the fingertip back and forth 10 times, and repeated after 2.5, 5.5 and 11 minutes in the same way as the tactile friction measurements. The samples were allowed to dry and the samples with dry residual films were attached to SEM aluminium sample stubs using Leit adhesive carbon tape (Agar Scientific) and sputtered with gold using an Agar automatic sputter coater at 30 mA, 0.08 mbar pressure with a sputtering time of 40 s. A conducting silver bridge was painted from the top of the sample down to the stub using Electrodag 1415 conducting silver paint (Agar Scientific). SEM micrographs of excised skin and VitroSkin® with and without dried formulation residues were obtained with a scanning electron microscope (Zeiss EVO LS10) equipped with a LaB6 filament. Imaging was done in high vacuum mode using a secondary electron detector, at 15 kV accelerating voltage, 50 pA probe current and 8-10 mm working distance.

3. Results and discussion

3.1 Influence of different untreated substrates on tactile friction

In order to compare the effects of using the two types of substrates, we first investigated the friction coefficient of the untreated substrates (no formulation applied), Fig. 1. As can be seen the friction coefficient is quite similar for the two different substrates and no significant difference was found ($p = 0.05$). However, the variation is as could be expected much larger for excised skin than for VitroSkin® and there is also a disparity between different porcine ears that occurs due to biological variation. To handle this variation, we propose to normalize studies on effects of topical formulations by dividing with the friction coefficient of the measurement on the pure untreated substrate and all data in the current work will be evaluated using this procedure.

Figure 1. The mean friction coefficient of untreated excised skin from different porcine ears A-G, horizontal stripes) and untreated VitroSkin® (diagonal stripes) measured by operator 1. Error bars show the standard deviation for n numbers of samples.

3.2 The effect of different operators on friction measurements

In order to further evaluate the method, we also compared the results obtained by two different operators on untreated substrates, Fig. 2. The individual variation for each operator was in the same range indicating that this is a systematic difference due to the method. There is a significant difference ($p < 0.05$) in measured friction obtained by the two operators when using untreated excised skin and VitroSkin®, although the difference in absolute friction values was smaller on VitroSkin®. The skin hydration (a.u) of the fingertip was higher for operator 2 than operator 1, (37 ± 18 vs 19 ± 5 with excised skin, 30 ± 6 vs 18 ± 3 with VitroSkin®), and it is tempting to consider that the higher skin hydration for operator 2 is the cause of the increased friction coefficients. Although differences in finger hydration is often the cause of large individual differences in tactile friction (Arvidsson et al., 2017; Derler and Gerhardt, 2012), it cannot be concluded that skin hydration of the fingertip is the only cause for difference in the results obtained by the two operators. The difference can probably also be attributed to a difference in finger size and individual response in applied load by the operators (Fig. S1, supplementary data), which shows that to be able to perform comparisons with exact values of friction coefficients using this method the same operator should conduct the full study. Other studies show that repeated measurements with one operator can be representative of relative differences in tactile friction between a set of samples (Arvidsson et al., 2017). Variations in

the relative humidity of the room (relative humidity = 43.7 ± 10.3 %, temperature = 21.3 ± 0.8 °C) had no systematic effect on the obtained friction coefficients for untreated substrates.

Figure 2. Variation between operator 1 (white bars) and 2 (grey bars) when using untreated excised porcine skin (horizontal stripes, $n = 6$) and VitroSkin® (diagonal stripes, $n = 6$) for tactile friction measurements. The data is shown as mean values with error bars showing the standard deviation for n numbers of samples.

In order to investigate how repeated measurements over time affect the results, the normalized friction coefficient for untreated substrates was investigated over a period of 11 minutes by operator 1, Fig. 3. The difference between VitroSkin® and excised skin small when investigating untreated substrates. However, excised skin shows a small reduction ($p < 0.05$) in normalized friction coefficient over the measurement period, there there was also a tendency for reduction in skin hydration (relative change %) for most untreated skin substrates after 11 minutes, $27\% \pm 34\%$ ($n = 8$). Even if variation in hydration was large, this may explain the small reduction in friction coefficient. Corresponding results for VitroSkin® show no significant change in the normalized friction coefficient over the measurement period.

Figure 3. Effect of time on the normalized friction coefficient for excised porcine skin ($n = 8$, horizontal stripes) and VitroSkin® ($n = 4$, diagonal stripes). Data is given as mean values with error bars showing the standard deviation for n numbers of samples.

3.3 Influence of topical creams on friction measurements on different substrates

To investigate if VitroSkin[®] resembles excised skin when measuring the influence of topical products on normalized friction coefficient, three different products were investigated, namely, Pickering cream, Canoderm[®] cream and a surfactant-based Pickering replica referred to as Surfactant cream (see Table 1 for composition). These creams have previously been shown to provide different tactile friction behaviour when measured on excised skin (Ali et al., 2021). Pickering cream and Surfactant cream have similar composition of ingredients and only differ in the type of emulsifying agents used, starch particles in Pickering cream and traditional surfactants in Surfactant cream.

During the application of a topical cream, the tactile friction is affected by the viscosity and thickness of the formed film between finger and substrate (Guest et al., 2013; Skedung et al., 2016; Tang et al., 2015). The frictional properties at this stage were thought to be related to slipperiness of the formulation (low friction = high degree of slipperiness) and the change over time was thought to reflect properties of the cream and the residual film on skin (ability to recover a normalized friction coefficient close to 1).

The difference between the previous results on excised skin (Ali et al., 2021) and the same measurements on VitroSkin[®] can be seen in Fig. 4. In this figure we have also added the measurements of operator 2 for Canoderm[®].

The trends seen previously for these creams on excised skin was also to some extent seen for VitroSkin[®], but with some notable differences. Firstly, the initial drop in normalized friction coefficient observed for Canoderm[®] and Surfactant cream was considerably lower on excised skin than on VitroSkin[®]. Secondly, while there was a small gradual increase in friction over time for Canoderm[®] and Surfactant cream on excised skin, on VitroSkin[®] the increase at 2.5 minutes that levelled out to close to unity for the remaining time (*i.e.*, same as untreated substrate). The normalized friction coefficients of Pickering cream were higher than for Canoderm[®] and Surfactant cream, and the increase of the normalized friction coefficient was more pronounced on VitroSkin[®]. The variation in the results between replicates was especially distinct for Pickering cream on excised skin and therefore it was difficult to evaluate the change over time as there seems to be at least an initial increase of the normalized friction coefficient over time.

Figure 4. Normalized friction coefficient over time for excised porcine skin and VitroSkin[®] at 32 °C. The tested creams were Canoderm[®], Pickering cream (bar with spheres) and Surfactant cream (dotted bar) and. The test for Canoderm[®] was performed by operator 1 (white bar) and 2 (grey bar). All other measurements were done by operator 1. Data are shown as mean values with error bars showing standard deviation for n = 3 number of samples.

It can be concluded that the gradual changes over time seen for excised skin were not observed for VitroSkin[®]. There could be several reasons for this. Firstly, the topography is not identical for the two substrates as seen with electron microscopy (Fig. 5, images A-B), hence the effect of spreading the creams could reduce friction more on the irregular excised skin than on the

slightly smoother VitroSkin®. Secondly, oil is unlikely to be absorbed by VitroSkin® while oil is expected to be absorbed to some extent by excised skin, which could explain the larger changes over time for the latter substrate. It can especially be realized for Canoderm® and Surfactant cream on excised skin where the gradual change over time reflects the spreading of a cream leading to a reduction of the normalized friction coefficient because of hydrodynamic lubrication. Subsequent reformulation and absorption of the residual oil lead to smoothening of the skin surface and a slow recovery of the normalized friction coefficient. This indicates that after the initial application, the residual film is in a mixed lubrication regime on excised skin for the remaining measurement time. On VitroSkin® however, the quick recovery in friction after spreading of Canoderm® and Surfactant cream reflects the inability of VitroSkin® to absorb the residual oil and thus no subsequent change in friction could be observed from the occlusive oil film. This indicates that after spreading, the thickness of the residual film is reduced exposing the peaks in VitroSkin®, and it is primarily these peaks that give rise to the friction in the boundary lubrication region (Fig. 4). The results for Canoderm® on VitroSkin® are in agreement with previous studies by Skedung et al. (Skedung et al., 2016) for a cream with similar composition, where it was also assumed that hydration resulted in further increase in friction coefficient over a longer measurement time (60 min). Similar observations have been made by Nacht et al. (Nacht et al., 1981) where the friction was initially reduced after application of greasy compounds, followed by gradual increase in friction over several hours. It must be kept in mind that although hydration and occluding effects might affect the tactile friction over longer time, the focus of this paper is on the perception of topical creams during and shortly after application.

The difference previously seen between the two operators for untreated substrates can now be compared to results from Canoderm® applied on excised skin and VitroSkin®. It was interesting to see if normalization of the results could reduce the differences previously seen for untreated excised skin. The difference in the results obtained by the two operators was small for excised skin in the initial measurements but over time the difference increased (Fig. 4). With VitroSkin®, there was an initial significant difference ($p < 0.05$) in results generated by the two operators, but after the first time point this difference was not significant and the operators obtained very similar normalized friction over time. We can conclude that although the operators obtained different results in tactile friction on untreated substrates, the difference between operators was rather small when topical formulations were applied. In general, one could assume that the difference in normalized friction is lower than the difference in absolute values.

It can be questioned whether changes over time as a result of reformulation and occlusion can be evaluated using VitroSkin®, whereas it could also be used to study changes over time as a result of hydration. Furthermore, the smaller variations between replicates and reproducibility of the results with VitroSkin® is beneficial from a statistical point of view to show direct differences between different types of topical creams. It can be concluded that although excised skin and VitroSkin® showed the same trends and that if one would rank the three creams the two substrates would give similar ranking, there were also considerable differences between these substrates.

3.4 Residual films at substrate surfaces

To further investigate the cause of the differences in results obtained with two substrates, we used scanning electron microscopy (Fig. 5). In line with previous studies, it can be seen that the topography and structure of untreated VitroSkin® imitates that of skin with peaks and valleys (Tang et al., 2015) while the peaks in VitroSkin® look more edgy compared to the untreated excised skin (Fig. 5, images A-B). The texture of VitroSkin® becomes more elastic and soft when hydrated as indicated by images shown by Tang et al. (Tang et al., 2015). Furthermore, untreated VitroSkin® contains randomly distributed small particles in the valleys but they could be distinguished from starch particles by the shape and unique particle size of starch particles (1-3 µm). Fig. 5 (images C-H) shows that for all three creams, dry formulation residues on excised skin and VitroSkin® could be clearly detected. This means that when applying the creams on the substrates with a finger in similar way as for the tactile friction measurements over a period of 11 minutes, there was still a detectable residue left on the substrates. Ultimately that means that during and by the end of the tactile friction measurements, the residual films of all creams on the two substrates were in the boundary/mixed lubrication regime. The Canoderm® residue (Fig. 5 E-F) displays a smoothening of the excised skin and the VitroSkin® surfaces, although the Canoderm® residue also shows some minor microstructure. The Surfactant cream residue (Fig. 5 G-H) shows more film coverage on both surfaces and exhibits similar smoothening of the surfaces as Canoderm®. The residual film of Pickering cream (Fig. 5 C-D) looks thicker than that of Canoderm® and Surfactant cream on both substrates. It is well distributed on VitroSkin® with starch and residual oil covering the surface, also creating differently sized aggregates of starch and oil locally. However, on excised skin the Pickering residue was not as well distributed with some areas covered by starch and oil, while other parts were not covered by the residual film. Furthermore, free starch could be seen on the excised skin while on VitroSkin® the starch is well distributed in the oil. The presence of starch in the residual film on VitroSkin®, and the aggregates of starch in the residual oil, could possibly increase the friction when the aggregates are rubbed against the edgy peaks. By comparing the residual films of Pickering cream (Fig. 5, images C-D) to Surfactant cream (Fig. 5, images G-H) it can be easily realised that the reason for the higher friction exhibited for Pickering cream is the presence of starch particles.

3.5 Influence of emollient and starch composition of Pickering emulsions

In order to further study the difference between excised skin and VitroSkin® as substrates and the mechanism behind the higher friction seen for Pickering cream, plain Pickering emulsions with different amounts of oil were investigated. The impact of the amount of oil on friction was studied by varying the triolein content in Pickering emulsions distributed on excised skin (Fig. 6), and a comparison between excised skin and VitroSkin® is shown in Fig. 7. The amount of starch added to these formulations, to ensure stabilization of emulsion droplets, is proportional to the amount of oil (300 mg starch/g oil), thus the starch content increases with increasing oil content in these emulsions.

Figure 5. SEM micrograph of dry residual films of applied creams on excised skin (left) and VitroSkin[®] (right). From top to bottom: Untreated skin samples (A-B), Pickering cream (C-D), Canoderm[®] (E-F), surfactant-based cream (Surfactant cream) (G-H). Scalebar is 20 μm .

Figure 6. Tactile friction measurements on Pickering emulsions with varying triolein oil content (and starch concentration 300 mg/g oil) on excised skin: 5 wt%, 10 wt%, 18 wt%, and 26 wt% oil (bars coloured with grey contrast from light to dark for low to high oil content, with white texture from low to high amount of starch). The test with Pickering cream with 28 wt% oil (white spheres) was also included for comparison.

The normalized friction coefficients of Pickering emulsions displayed in Fig. 6 differed the most during application. Lower oil content (5-10 wt%) did not induce a reduction of the friction coefficient compared to untreated skin, while higher oil content (18-26 wt%) resulted in hydrodynamic lubrication and thus a reduction in friction. Further measurements resulted in a similar or higher friction coefficient compared to untreated skin. The emulsion with 18 wt% oil significantly differed ($p < 0.05$) from the one with 10 wt% oil during the application as well as at 5.5 minutes. In previous studies with traditional surfactant-based emulsions it has been shown that the friction on skin decrease due to the lubrication of the applied oil layer (Savary et al., 2013). As can be seen for the Pickering emulsions this is only the case when the oil content is high and contrary to previous investigations the recovery of the friction is quite fast. In addition, Pickering creams are often described as dry and ranked low for greasiness and stickiness (Ali et al., 2021) and this is in line with a high friction coefficient. After 11 min, the friction coefficients of all emulsions were similar regardless of oil content. Only the emulsion with 10 wt% oil was significantly different from the three other emulsions ($p < 0.05$). When the results of the emulsions were compared with the results of the Pickering cream, the statistical analysis found no significant differences ($p = 0.05$) between the samples. This suggests that the residual film structure is similar for Pickering emulsions irrespective of oil content after a while when the oil has been rubbed into the excised skin. For Pickering emulsions with 5 wt% triolein, the change in friction coefficient is similar on excised skin and VitroSkin[®]. However, with higher oil content (26 wt%) the difference in friction change is much higher on VitroSkin[®] than on excised skin (significantly different from 5 wt%, ($p < 0.05$)). The difference in frictional behaviour observed between Pickering cream and the Pickering emulsions can be attributed to the overall structure of the cream due to addition of other excipients.

Figure 7. Normalized friction coefficient of applied Pickering emulsions with varying triolein oil content (and starch concentration 300 mg/g oil) on excised skin and VitroSkin®: 5 wt% (light grey bar with white texture) and 26 wt% oil (black bar with white texture). The tests with Pickering cream (emulsion with other excipients) with 28 wt% oil (white spheres) are also included for comparison.

3.6 A closer look at the residual films at surfaces

The results suggest that VitroSkin® is more sensitive to presence of particles than excised skin. It can be speculated that although VitroSkin®, when hydrated, imitates the topography of excised skin, it lacks some specific features such as the wrinkles and hair follicles that are present on porcine and human skin. This difference may well account for an extra sensitivity to small particles in formulations on VitroSkin® and an initial higher tactile friction when compared to excised skin. As the water evaporates over time, the surface of VitroSkin® becomes rough and stringent resulting in higher tactile friction when the residual starch-oil film is further rubbed with the finger on this surface. Thus, on VitroSkin® the friction increases as a result of higher particle content in combination with high degree of aggregation of these particles with the residual oil. Consequently, this increase in friction is more prominent and takes over the decrease in friction normally seen for higher oil content. In comparison, excised skin can be thought to have a softer structure than VitroSkin® over time, and the presence of wrinkles and

hair follicles could explain why the impact on tactile friction of different particle content in formulations is barely noticed. Consequently, particles are well distributed on the skin surface and contributing to a friction similar or slightly higher than untreated skin regardless of oil content. This can also be seen in the SEM image of 26 wt% oil Pickering emulsion (Fig. 8B) where the particles seem to be well distributed over the skin surface. Furthermore, the distribution of starch particles in the residual film differs between excised skin and VitroSkin[®], with more freely distributed starch on excised skin, whilst being particles are more tightly packed on VitroSkin[®]. This could be a result of non-absorbed residual oil on VitroSkin[®] creating a paste-like film with the starch on the surface, while on excised skin some of the oil is absorbed leaving free starch particles on the top. This correlates well with our previously reported *in vivo* data (Ali et al., 2021), where a Pickering cream was perceived as non-greasy in the after-feel. For Pickering creams, it has sometimes been observed that further substantial rubbing during the application phase may cause the residual formulation to peel off from the skin as in a “snowball effect”. Similar “peeling” of the residues was observed in the tactile friction measurements of Pickering cream on VitroSkin[®] (mainly at 2.5 min) but not for excised skin. Thus, it could be hypothesised that the mechanism for these phenomena could be the same. That is, that residual excipients, such as oil and/or thickener, in combination with the starch particles form aggregates, that are peeled off the skin upon further rubbing, when the oil is not absorbed fast enough into the skin. Therefore, VitroSkin[®] could be suitable for studying this type of phenomena, as it could possibly reflect skin types where oil absorption does not occur fast and are more prone to experience this phenomenon.

Figure 8. SEM micrographs showing residual film of Pickering emulsion (26 wt% triolein) on excised porcine skin (A) and VitroSkin[®] (B).

4. Conclusions

It can be concluded that there are no significant differences in obtained friction coefficient on untreated excised skin and VitroSkin[®] for a trained operator. The variation was, however, much larger for excised skin than for VitroSkin[®]. The measured friction obtained individually by the two operators differed significantly on both untreated substrates, and the variation was more substantial for excised skin than for VitroSkin[®]. When a topical cream was applied the difference between operators was however rather small. To study significant differences between products with controlled measurements, a single trained operator is to be preferred. We can further conclude that when measuring on untreated substrates, the tactile friction depends on the properties of the substrate surface. Once a formulation is applied, it is the physicochemical properties of the formulation that determines the results in tactile friction. The effect of measuring time was rather small both for untreated excised skin and VitroSkin[®]. On the other hand, when evaluating formulations, time effects are seen to some extent for VitroSkin[®], but to much larger extent for excised skin. Nonetheless, we can conclude that

excised skin and VitroSkin® showed the same trends when formulations are applied, and that if one would rank two creams towards each other the two substrates would give similar ranking. The difference between excised skin and VitroSkin® could be attributed to surface structure differences, as demonstrated by SEM. While VitroSkin® fails to reproduce the topical properties of excised skin that affects tactile friction over time, it can be used to detect direct differences in friction between topical products. It can be a suitable substitute to avoid using animal or human skin, and to reduce the individual variation between skin substrates due to biological variation. Furthermore, it can be used to study specific features such as hydration effects, and “peeling” effects of particle-containing topical products.

The results also confirmed that there is a difference between Pickering-based formulations and surfactant-based formulations such as Surfactant cream and Canoderm®, indicating that surfactant-based creams are greasier than the Pickering cream shortly after application. It was also shown that the amount of oil only has a minor effect on the friction for Pickering emulsions. Friction is consistently high even at high amounts of oil indicating a non-greasy, and non-sticky formulation in line with previous results by Ali et al. (Ali et al., 2021). Thus, we can conclude that starch-stabilized Pickering formulations present unique characteristics allowing the development of novel surfactant-free topical formulations with specific tactile properties of the afterfeel perception.

5. Acknowledgements

We are grateful to the Knowledge foundation (Sweden) for funding the project, and Johan Engblom also thank the Gustaf Th Olsson foundation (Sweden) for financial support. Speximo AB is acknowledged for providing starch particles.

6. Conflict of interest

The authors declare no conflict of interest.

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Supplementary Information

Figure S1. Average applied load for operator 1 (white bars) and 2 (grey bars) when using untreated excised porcine skin (horizontal stripes) and VitroSkin® (diagonal stripes) for tactile friction measurements. The data is the average of 6 different measurements.

III

Alcohol-based Pickering emulsions as moisturizing topical sanitizers

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Key words: Pickering emulsion, Alcohol-based hand rub, Disinfection, Hand sanitizer, Skin dehydration.

Abstract

Frequent hand washing and use of alcohol-based hand rubs is essential for keeping good hand hygiene and for the prevention of spreading disease. People who use hand hygiene products frequently, such as health-care workers, tend to suffer from dry hands and irritant contact dermatitis which can lead to bad compliance to hand hygiene routines. There is clearly room for improvement for hand hygiene products to increase compliance. In this project we aimed to evaluate the potential use of the Pickering emulsion concept to create a surfactant-free, user-friendly, and moisturizing alcohol-based hand rub.

We approached this by choosing two triglycerides with different physicochemical properties, tricaprin and triolein, and mapping their phase behavior in the ternary triglyceride-ethanol-water system. Thermogravimetric Analysis, Differential Scanning Calorimetry, X-ray diffraction, Optical Microscopy, as well as Scanning Electron Microscopy were employed to further characterize the system and the ability of quinoa starch particles to stabilize an alcohol-containing emulsion. Tactile friction studies and evaluation by a human panel were employed to characterize the perception of touch and user acceptance of the formulations.

We conclude that it is possible to create alcohol-based Pickering emulsions as potential moisturizing alcohol-based hand rubs with sensorial preference over conventional alcohol-based hand rubs. The stability of the emulsions was confirmed after 3 years of storage at room temperature. The oil phase preferentially comprises triglycerides with long unsaturated fatty acid chains to minimize miscibility of the polar and the non-polar phase while maintaining a melting point for the triglyceride at or below skin surface temperature.

1. Introduction

Frequent hand washing with soap and water and use of alcohol-based hand rubs is essential for keeping good hand hygiene and is the most important measure to prevent spread of infectious diseases and contagious illnesses (1,2). Most alcohol-based hand rubs are either solutions or gels containing either ethanol or isopropanol, or a combination of the two for a synergistic effect (2). The antimicrobial activity of alcohols results from their ability to denature proteins (3–5). Ethanol concentrations of 60 to 95 vol% are deemed safe and effective for disinfection by the United State Food and Drug Administration (US FDA) (6), and World Health Organization (7). According to the WHO, alcohols have an excellent activity against Gram-positive bacteria, Gram-negative bacteria, enveloped viruses, non-enveloped viruses, mycobacteria, and even fungi (7).

The importance of alcohol-based hand rubs has been accentuated by the rapid spread of the global pandemic, CoVid-19. To reduce the risk of viral transmission the Center for Disease Control and Prevention (CDC) suggests frequent hand washing with soap and water for 20 seconds or use of alcohol-based hand rubs with at least 60% alcohol (8). This led to an increase in sales of hand sanitizer products during the first quarter of 2020 (9) and the use of alcohol-based hand rubs have increased exponentially leading to a rise in reported incidence of hand eczema (10,11). It has caused major concerns in the dermatology community calling for extra precautions and recommending use of alcohol-based hand rubs with humectants and use of skin care products (12–15). For health-care workers, hand washing with soap and water together with hospital routines requiring up to 40 daily alcohol-based hand rub disinfections is a severe problem (16). Irritant contact dermatitis has been reported to be extremely common among health-care workers, even before the pandemic (17,18). Increased awareness of alcohol-based hand rub among consumers will eventually generate ethical demands such as use of natural and sustainable ingredients and raise health issues regarding skin tolerance of surfactants. Taken together with sensorial preferences, ethical and health issues will influence compliance.

Skin damage by hand hygiene products involve denaturation of stratum corneum proteins, changes in intercellular lipids, decreased corneocyte cohesion and decreased stratum corneum water binding capacity (19,20). Among these the main concern is the depletion of the lipid barrier that may be a consequence on contact with lipid emulsifying detergents and lipid-dissolving alcohols, both contributing to barrier dysfunction (21). Although alcohol-based hand rubs are considered safer than handwashing with detergents (22–30), there is still a general belief and concern that they can cause dryness, skin irritation, and consequently frustrate compliance (1–3,12,16,26,31–34). Glycerol is commonly used in alcohol-based hand rubs to promote hydration and user acceptance, but it has been reported to slow down drying time and convey a sticky sensation (16,35–37). There is a consensus that in order to maintain skin health, an emollient-containing skin moisturizer should be used after hand washing and disinfection (12,13,15,38,39).

To avoid skin irritation caused by surfactants, we propose an innovative solution by using Pickering emulsions to create a skin-moisturizing alcohol-based hand rub in the form of a cream or lotion. The emulsion would offer moisturization and skin care with an emollient, along with an antimicrobial effect. In Pickering emulsions, the emulsifiers are replaced by solid colloidal particles, such as starch granules, partly wetted by the oil and water, and physically stabilizing oil-water interfaces by adsorbing to the liquid-liquid interface (40). Previous studies on Pickering emulsions stabilized with quinoa starch demonstrated long-term stability against coalescence and Ostwald ripening (41), and unique sensory properties after application to the skin (42,43). To create such a product there are some important aspects to resolve. Recently we have shown that alcohol-based Pickering emulsion are antimicrobial (44). However, a hand cream is generally an oil-in-water emulsion, and since ethanol is miscible with water and dissolves some common oils, the alcohol may interfere with the liquid-liquid interface of such an emulsion. Furthermore, dissolution of oil in the hydrophilic phase may contaminate the alcohol and reduce the antimicrobial efficacy.

The aim of this study was to evaluate an alcohol-based Pickering emulsion as a skin-moisturizing alcohol-based hand rub. Three main questions were identified:

- a. Is it possible to create alcohol-based Pickering emulsions with quinoa starch?

- b. Will the oil dissolve in the ethanol-water phase and thus risk to impede its antimicrobial effect, or will ethanol alternatively dissolve in the oil phase and change its physicochemical properties?
- c. Will an emollient-containing alcohol-based hand rub result in higher user compliance than liquid ethanol products?

It is important to understand the limitations in composition for such a system including effects of ethanol as a solvent on the physicochemical properties of starch, and on the liquid-liquid interface in the emulsions.

2. Materials and methods

2.1. Materials

Ethanol (99.8%) was purchased from VWR Chemicals (Stockholm, Sweden), while tricaprins (Captex 1000, 98.5% purity) and triolein (Captex GTO, 90.2% purity) were obtained from Abitec Corporation (Janesville, USA). Glycerol was purchased from Sigma-Aldrich (Stockholm, Sweden). Modified quinoa starch, used as Pickering particles, was kindly provided by Speximo AB (Lund, Sweden). Ultrahigh quality (UHQ) water, purified at 25 °C by the Elgastat UHQ II Model UHQ-PS-MK3 (Elga Ltd., High Wycombe, Bucks, UK) was used throughout the study. The artificial skin, Vitro-Skin[®], used in friction measurements was purchased from IMS Inc. (USA). Commercially available ethanol-based hand sanitizers, Ethanol Hand Disinfectant (Sterisol AB, Sweden) and Alcogel 85 (Dax, Sweden), were sourced from a local pharmacy. Ethanol Hand Disinfectant contained 70 wt% ethanol and 10 wt% isopropyl alcohol as active ingredients, glycerin as humectant, and carbomer as thickener, while Alcogel 85 contained 85 wt% ethanol as active ingredient, glycerin as humectant, and carbomer as thickener.

2.2. Preparation of Pickering emulsions

Pickering emulsions were prepared with either of the two triglycerides, tricaprins (TC) or triolein (TO), as the dispersed oil phase and ethanol-water solutions as the continuous phase. Modified quinoa starch, 400 mg starch per ml oil, was added to all emulsions as an emulsion stabilizer. A range of compositions were prepared by varying the ratio between dispersed phase and continuous phase and varying the ethanol/water concentration in the continuous phase. The compositions of the emulsions are listed in Table 1.

Starch was added and vortexed with the continuous phase in a glass container for one minute before adding the triglycerides and vortexing for another minute followed by emulsification with a high shear mixer (IKA Ultra-Turrax T25) for 1-2 minutes at 18000-22000 rpm. The sample containers were photographed to follow the emulsion stability with time.

2.3. Preparation of triglyceride-ethanol-water mixtures

The liquid triglycerides, tricaprins at 40 °C, and triolein at room temperature, were mixed with ethanol-water solutions, comprising 55 wt%, 70 wt%, 85 wt%, and pure (99.8 wt%) ethanol, in the following triglyceride-ethanol solution weight ratios: 30:70, 50:50, and 70:30. For simplicity, ethanol-water solutions will be hereafter referred to as ethanol solutions together with the specific concentration. The samples were then left to equilibrate in the dark in flame-sealed glass ampoules at 25 °C and 40 °C reflecting relevant stability study temperatures. The sample set is summarized in table 2. All samples were photographed directly after mixing, 24 hours after preparation and weekly during storage for 8 weeks (50:50 samples) and 2 weeks (30:70 and 70:30 samples). Macroscopic phase behavior, including

phase separation, was noted and ampoules were then opened to extract individual phases for further analysis. Exact sample composition and phase boundaries in the ternary triglyceride-ethanol-water mixtures are tabulated in Table S1 in Supplementary data.

2.4. Preparation of starch dispersions in ethanol solutions

Starch dispersions were prepared by mixing quinoa starch particles with ethanol solutions to obtain a 16 wt% starch dispersion in 20 ml glass bottles. The mixtures were vortexed one minute and homogenized with an Ultra Turrax at 15000 rpm for minute to disintegrate starch aggregates. Starch dispersions (16 wt%) for DSC measurements were vortexed in Eppendorf tube for one minute and left for at least one hour at room temperature (22 °C) before performing measurements.

Table 1. Composition of Pickering emulsions (PE) with triglycerides Tricaprin (TC) or Triolein (TO). The emulsions were screened by varying the ratio between dispersed phase and continuous phase, as well as the ethanol concentration (wt%).

Sample name	Dispersed phase (wt%) Triglycerides (TC/TO)	Continuous phase (wt%) (H ₂ O/EtOH)	Quinoa starch (wt%)	Dispersed: Continuous phase ratio	Ethanol in continuous phase (wt%)	Ethanol in total sample (wt%)
PE TC/TO:H ₂ O (7:3)	53	23	24	30:70	0	0
PE TC/TO:H ₂ O (5:5)	41	41	18	50:50	0	0
PE TC/TO:H ₂ O (3:7)	26	62	12	70:30	0	0
PE TC/TO:H ₂ O (2:8)	18	74	8	80:20	0	0
PE TC/TO:H ₂ O (1:9)	10	86	4	90:10	0	0
PETC/TO:H ₂ O (0.5:9.5)	5	93	2	95:5	0	0
PE TC/TO:55% EtOH (7:3)	53	23	24	30:70	55	12.6
PE TC/TO:55% EtOH (5:5)	41	41	18	50:50	55	22.6
PE TC/TO:55% EtOH (3:7)	26	62	12	70:30	55	34.0
PE TC/TO:55% EtOH (2:8)	18	74	8	80:20	55	40.4
PE TC/TO:55% EtOH (1:9)	10	86	4	90:10	55	47.4
PE TC/TO:55% EtOH (0.5:9.5)	5	93	2	95:5	55	51.1
PE TC/TO:70% EtOH (7:3)	53	23	24	30:70	70	16.0
PE TC/TO:70% EtOH (5:5)	41	41	18	50:50	70	28.8
PE TC/TO:70% EtOH (3:7)	26	62	12	70:30	70	43.2
PE TC/TO:70% EtOH (2:8)	18	74	8	80:20	70	51.5
PE TC/TO:70% EtOH (1:9)	10	86	4	90:10	70	60.4
PE TC/TO:70% EtOH (0.5:9.5)	5	93	2	95:5	70	65.1
PE TC/TO:85% EtOH (7:3)	53	23	24	30:70	85	19.5
PE TC/TO:85% EtOH (5:5)	41	41	18	50:50	55	34.9
PE TC/TO:85% EtOH (3:7)	26	62	12	70:30	55	52.5
PE TC/TO:85% EtOH (2:8)	18	74	8	80:20	55	62.5
PE TC/TO:85% EtOH (1:9)	10	86	4	90:10	55	73.3
PE TC/TO:85% EtOH (0.5:9.5)	5	93	2	95:5	55	79.0

2.5. Optical Microscopy

Pickering formulations were analyzed by optical microscopy (20x magnification) to evaluate emulsion droplet size and emulsion stability, using a Nikon Optiphot microscope equipped with a DS-U1 digital camera. 1-2 drops of formulation were diluted (5x) with water and spread on a glass-slide, no cover glass was used.

2.6. Differential Scanning Calorimetry (DSC)

The thermal behavior of the triglyceride fraction of phase separated samples in the triglyceride-ethanol-water system was studied with DSC (DSC1, Mettler Toledo Inc., Switzerland) to elucidate if ethanol has any impact on the melting properties of the triglycerides. The extracted triglyceride fractions were first cooled down to -60 °C, kept for two minutes at that temperature and then heated to 60 °C and kept at that temperature for two minutes. The measurements were performed as two subsequent heat scans. For comparison, pure triglycerides were also analyzed. The onset temperature, T_{onset} , and the endset temperature, T_{endset} , (defined as the point where the first tangent (T_{onset}) and the second tangent (T_{endset}) of the peak intersect with the extrapolated baseline), and the enthalpy of melting, ΔH_m , (defined as the integrated endothermic peak) was calculated for each sample and averaged for different triglyceride compositions (3:7, 5:5, and 7:3) mixed with the same ethanol concentration, see Table 2. Measurements on tricaprins samples stored at 25 °C and 40 °C were handled as replicates of each other, as they should behave the same when the solid oil phase is separated from the ethanol-water phase and extracted to a new vial at room temperature.

The gelatinization temperatures of quinoa starch in different ethanol-water solutions were determined by heating the samples from 25 °C to 95 °C to record the endothermal gelatinization process. The peak temperature, T_p , and the gelatinization transition enthalpy, ΔH_g , was calculated for each sample.

DSC was also used to analyze triglyceride-ethanol-water mixtures and starch dispersions in ethanol-water solutions respectively. The samples (10-15 mg) were transferred to 40 μl aluminum crucibles with pins (Mettler Toledo) and directly hermetically sealed, to avoid solvent evaporation, and placed in the DSC-instrument. All cooling and heating segments were run with a scan rate of 10 °C min^{-1} and a nitrogen flow of 80 ml min^{-1} . Indium was used as a calibrant and an empty aluminum crucible (40 μl) was used as reference. Replicate samples were measured for each composition, to confirm the reproducibility of the DSC experiments. The DSC data was evaluated and integrated using the STARE software (version 16).

2.7. Thermogravimetric Analysis (TGA)

Thermogravimetric analysis (TGA) was primarily used to determine the amount of triglyceride (*i.e.*, triolein and tricaprins, respectively) that may have dissolved into the ethanol-water phase in samples suffering phase separation. The weight loss of the samples studied was determined as a function of time and temperature using a TGA Q500 (TA Instruments, USA). By combining the measured weight content remaining at a certain temperature and known boiling points for each constituent, TGA could be used to determine the content of each constituent in the studied samples. Samples were transferred with a plastic pipette to a platinum pan. The starting temperature was 25 °C for triolein samples, and 25 °C or 40 °C for tricaprins samples stored at the different temperatures. The samples were kept at the starting temperature for one minute, followed by heating at 10 °C min^{-1} until 600 °C was reached. At 600 °C the temperature was kept for five minutes before stopping the measurement.

Following the TGA curves for mass loss as a function of time and temperature, the residue representing the triglyceride content in the sample could be determined at 100 °C after ethanol and water had evaporated. The amount of triglyceride in the oil phase was averaged for samples with different triglyceride contents along the anticipated tie-line.

2.8. Drawing of ternary phase diagrams

The concentration of triglycerides in the triglyceride fraction, “w_{TG}(TG)”, was calculated based on the lever rule for values taken from TGA analysis on the ethanol-water fraction and the volume fractions of the triglyceride-ethanol-water phases. Based on this, ternary phase diagrams for the triglyceride-ethanol-water systems were constructed using the values presented in Table S1 (Supplementary data). To be able to construct the phase diagram a couple of assumptions were made. We assume that the ethanol:water ratio remains constant for all ethanol solutions. Furthermore, we assume that the samples mixed with the same ethanol concentration dissolve similar amount of triglyceride. In addition, for each tie-line in the two-phase region, an average of the samples with different triglyceride composition and fixed ethanol concentration was calculated as given in Table 2. When it was difficult to distinguish the volume fractions in the samples, the samples were excluded from the phase diagram.

2.9. Small and wide-angle X-ray diffraction (SWAXD)

All SWAXD experiments were carried out on a SAXD/WAXD instrument (a Xeuss 3.0 from Xenocs, at Malmö University, Sweden) using a CuK_α-radiation ($\lambda = 1.542 \text{ \AA}$) source with a PILATUS3 R 300K detector. Measurements were carried out under vacuum using a temperature control Peltier Stage. The samples (Pickering emulsions and starch dispersions) were pipetted into gel-holder sample cells, with an O-ring as the spacer between two Kapton films (DuPont™Kapton®, 0.013 mm thickness, Goodfellow, England) or two mica sheets.

Two sample detector distances, 70 mm and 1700 mm, were used to collect the WAXS and SAXS data respectively, covering a total q-range of $0.0002 \leq q \leq 3.1 \text{ \AA}^{-1}$. The scattering vector, q , was defined as $|q| = q = \frac{4\pi}{\lambda} \sin \frac{\theta}{2}$, in which θ is the scattering angle and λ is the wavelength of the X-ray source. The data in the range of $0.0002 \leq q \leq 0.15 \text{ \AA}^{-1}$ were used as the SAXS region, and data in the range of $0.15 \text{ \AA}^{-1} \leq q \leq 3.1 \text{ \AA}^{-1}$ were used as the WAXS region. The exposure time for SAXS data was collected with 5 x 300 s (frames x exposure time), and for WAXS data 10 x 60 s to control for any sample leakage. Data was collected at 25 °C, 40 °C and 60 °C. Data reduction, normalization, summing and 1D azimuthal averaging were done using Xenocs software XSACT 2.4.

2.10. Scanning Electron Microscopy (SEM)

Samples were prepared by applying and spreading starch dispersions (16 wt%) on VitroSkin® with a gloved fingertip. When the samples were dry, the VitroSkin® with dry starch films were attached to SEM aluminium sample stubs using Leit adhesive carbon tape (Agar Scientific) and sputtered with gold using an Agar automatic sputter coater at 30 mA, 0.08 mbar pressure with a sputtering time of 40 s. A conducting silver bridge was painted from the top of the sample down to the stub using Electrodag 1415 conducting silver paint (Agar Scientific). SEM micrographs were recorded using a Zeiss EVO LS10 scanning electron microscope equipped with a LaB6 filament. Imaging was done in high vacuum mode using a secondary electron detector, at 15 kV accelerating voltage, 50 pA probe current and 8-10 mm working distance.

2.11. Tactile friction measurements

Tactile friction measurements were performed using a ForceBoard™ (Industrial Dynamics Sweden AB, Järfälla, Sweden), following the method described by Skedung et al. (45,46) with minor adjustments. The friction force (F) and applied load (L) were continuously recorded as a finger interrogated the VitroSkin® surface by moving the index finger back and forth, and the friction coefficients (μ) were calculated as the ratio of the friction force and load according to:

$$\mu = \frac{F}{L} \quad (1)$$

A temperature-controlled plate was placed on top of the ForceBoard® and the substrates were mounted on the heated plate (32 °C). Pre-hydrated VitroSkin® (2x5 cm²) was attached with double-adhesive tape. Before each experiment, a measurement on untreated substrate with 10 strokes was performed as control for changes in substrate and finger. For each experiment the index finger, inclined 30°, was stroked forward and back 10 times to spread 6-7 mg cm⁻² of formulation on the substrate over a sample area of approximately 7.5 cm².

The friction was measured for 30 seconds during application of the formulation and again after 2.5, 5, and 10 minutes without washing the index finger in between. Between each experiment, the hand was washed thoroughly with soap and allowed to dry 5 minutes before next experiment. All experiments were performed in triplicates and in controlled ambient conditions (Temperature = 22 ± 1 °C, relative humidity = 39 ± 13 %). The friction force and the applied load were recorded with a sampling rate of 100 Hz and the applied load was maintained around 1.0 N. The normalized friction coefficient was calculated by dividing the measured friction force for the topical formulations with the control measurement on untreated skin substrate. This means that a sample with a greater friction coefficient than 1 displays higher tactile friction than the untreated substrate.

2.12. Evaluation by human sensory panel

The evaluation of the sensory perception was carried out on four different topical formulations for perceptive attributes during application and afterfeel according to the protocol described by Ali et al. (43) with minor adjustments. The formulations were Ethanol Hand Disinfectant, PE TO:H₂O (3:7), PE TO:70%EtOH (3:7), and PE TO:70%EtOH (0.5:9.5).

Thirty healthy volunteers, eight men and twenty-two women, with no history of skin disease were recruited to participate as assessors in the sensory study in accordance with ISO 8586:2012. Participants ages ranged from 18 to 54, but most of the participants were in the 18-34 age group (93.5%). To conduct the study, ethical approval was received by the Sweden Ethical Review Agency (DNR 2019-05452) and informed consent was obtained from all the participants.

The sensory attributes were assessed on two defined circular measurement sites ($d = 4$ cm) on each volar forearm, placed 7 cm from the wrist and elbow, and distanced 2 cm from each other. *In vivo* baseline measurements of trans epidermal water loss (TEWL) for each measurement site were taken using a Delfin Vapometer (Delfin Technologies, Finland) prior to application of 30 µl of each formulation by the project leader to the middle of the circular measurement sites using a M1000 positive displacement pipette (Microman®, Gilson, France). A blank skin area on the inside of the right forearm was also measured at the same timepoints as a reference for each individual. The relative humidity in the air was 22-23% and the room temperature 21-22 °C. Upon completion of the study and removal of the formulations, another measurement was done (approximately 15 minutes after application) for all participants. For some participants additional measurements were taken, after 30 minutes, and after 45 minutes to investigate if there would be any difference.

Once the formulations were applied, the participants were allowed to start the evaluation following the instructions on a digital survey (Microsoft Forms) on a tablet computer. The study was divided between evaluation of attributes during application phase, and attributes during the afterfeel phase (10 minutes after application). After evaluating the attributes during application, the assessors were asked to clean their fingers with facial tissues and answer a general questionnaire during the waiting time before the afterfeel evaluation phase. The sensory attributes evaluated, and corresponding instructions are summarized in Table S2 (Supplementary data).

The formulations were ranked for each attribute according to the rank test (ISO 8586:2006) (47,48) ranking the four formulations from 1 to 4 for the highest to lowest perception for each specific attribute. After ranking the formulations, the assessors could choose their preferred formulation for each studied attribute. They were also asked to choose their overall preferred formulation during the application phase and between the formulations overall. By the end of the study, the assessors were also asked to describe each formulation by choosing words from a word cloud, or by using their own words. For the attributes “perception of particles”, “stickiness”, and “odour” the participants were first asked whether or not they perceived/sensed anything for any of the formulations. If they did, they could continue to rank the formulations based on perception. If not, they could skip ranking the formulations for this attribute.

2.13. Statistical Analysis

Whenever applicable, results were expressed as averages $\pm$ standard deviation, and differences between cream formulations were determined by one-way ANOVA with Tukey’s HSD test. Statistical outliers were excluded based on two-sided Grubbs’ test ($p < 0.05$).

A Friedman test of difference was used to determine whether participants had a significantly different rank ordered preference for the four cream formulations ($p < 0.05$). The Friedman test is the non-parametric alternative to the one-way ANOVA and shows whether significant differences exist between two or more samples that are ranked by all panelists. It is a very sensitive test to find a pattern of consistent rank order (49). Whenever there was significant difference, pairwise comparison was performed using Fisher’s least-significant-difference (LSD) test to determine which of the cream formulations were significantly different ($p < 0.05$).

3. Results and Discussion

3.1 Alcohol-based Pickering emulsions

Pickering emulsions with tricaprln or triolein could be produced for almost all the compositions in Table 1. In general, the emulsions were milky white with a lotion-like texture. The only samples which could not produce an emulsion were the samples with a dispersed:continuous phase of 7:3. This was due to the fact that the samples held large quantities of oil, and also a proportionally large amount of starch that absorbed the small quantity of aqueous liquid available resulting in grainy starch-oil mixtures. Water-based Pickering emulsions with dispersed:continuous phase ratios of 5:5 and 3:7 appeared as homogeneous white emulsions while other ratios lead to creaming of the dispersed phase. When the amount of triglyceride was decreased, the water-based emulsions became fluffy and floated on top of a water layer, see Figure S1 in Supplementary data. Alcohol-based Pickering emulsions (55-85 wt% ethanol in continuous phase) were produced for all dispersed:continuous phase ratios for both tricaprln and triolein.

Representative microscope images of emulsion droplets in tricaprln or triolein emulsions with fixed dispersed:continuous phase ratio (3:7) and varying amount of ethanol-water solutions in the continuous phase are shown in Figure 1, together with photos of corresponding bulk emulsion in glass container.. Particle stabilization could be confirmed for all emulsions with quinoa starch particles (1-2 μm) attached to the oil droplets. Droplet size of tricaprln emulsions was in general larger than for triolein emulsions.

Furthermore, the stability against coalescence of emulsion droplets could be confirmed for emulsions stored > 3 years at room temperature (23 °C, Figure 2).

The screening of Pickering emulsions with varying concentration of triglyceride and ethanol tends to show an effect of the solvent on the efficiency of emulsion stabilization and droplet size. As noted in Figure 1A, when water was used the starch particles were aggregated and the emulsion droplets packed together when water was used resulting in smaller oil droplets. When in ethanol solutions, in Figure 1B-C, the starch particles seemed to be more loosely distributed and the emulsion droplets were larger and less packed together. Droplet size increased gradually with increasing amount of ethanol in the continuous phase. There was a visible sedimentation of the dispersed phase for the ethanol-based emulsions. The larger oil droplets covered with starch (density of starch $\sim 1.5 \text{ g cm}^{-3}$), in combination with the low density of the ethanol solution, resulted in sedimentation of the emulsion part and a less viscous emulsion. These features were present for both triolein and tricaprln emulsions.

Having these observations in mind, it was interesting to investigate the impact of varying ethanol solutions on the individual triglycerides and starch separately and understand how it may affect the liquid-liquid interface and emulsion properties.

Figure 1. Light microscopy micrographs (20x magnification) showing oil droplets of Pickering emulsions (PE) with 3:7 ratio of dispersed phase to continuous phase for triolein (A-C) and tricaprln (D-F) emulsions after preparation. Photographs of corresponding bulk emulsion in glass container are attached. Triolein emulsions with water (A, droplet size $\sim 20\text{-}35 \text{ }\mu\text{m}$), with 55 wt% ethanol (B, droplet size $\sim 30\text{-}50 \text{ }\mu\text{m}$), and 70 wt% ethanol (C, droplet size $\sim 30\text{-}50 \text{ }\mu\text{m}$). Tricaprln emulsions with water (D, droplet size $\sim 25\text{-}50 \text{ }\mu\text{m}$), with 55 wt% ethanol (E, droplet size $\sim 40\text{-}70 \text{ }\mu\text{m}$), and 70 wt% (F, droplet size $\sim 50\text{-}90 \text{ }\mu\text{m}$). Scale bar is 50 μm .

Figure 2. Light microscopy micrographs showing the emulsions after > 3 years storage at room temperature. Triolein (A) and tricaprln (B) Pickering emulsions (PE) with 3:7 ratio of dispersed to continuous phase. Photographs of corresponding bulk emulsion in glass containers are attached. A: Triolein Pickering emulsions with 70 wt% ethanol with droplet size ~ 20-50 μm . B: Triolein Pickering emulsions with 70 wt% ethanol with large droplets ~ 70-100 μm . Scale bar is 50 μm .

3.2 The triglyceride-ethanol-water system

Ternary phase diagrams were constructed for tricaprln-ethanol-water at 25 $^{\circ}\text{C}$ (Figure 3A), at 40 $^{\circ}\text{C}$ (Figure 3B), and triolein-ethanol-water at 25 $^{\circ}\text{C}$ (Figure 3C). All triglyceride-ethanol-water mixtures (Table S2, Supplementary data), with a few exceptions, separated within two months when stored at 25 $^{\circ}\text{C}$ and 40 $^{\circ}\text{C}$. Tricaprln is fully soluble in ethanol at 40 $^{\circ}\text{C}$, and soluble up to 30 wt% at 25 $^{\circ}\text{C}$. Triolein-ethanol-water mixtures with 55 wt% ethanol had an emulsion-like cloudy appearance when the triolein to ethanol-water ratio was 3:7 (Table S2, Supplementary Information). The density of 55 wt% ethanol (0.90 g cm^{-3}) at 20 $^{\circ}\text{C}$ is very similar to the density of triolein (0.91 g cm^{-3}), which explains why triolein and the aqueous ethanol phases did not phase separate. Consequently, individual phases from these samples could not be extracted and no further analysis was done.

Tricaprln proved to be more soluble in ethanol than triolein. At 25 $^{\circ}\text{C}$, the phase diagram for tricaprln-ethanol-water shows a larger one phase region in the ethanol corner. The one phase region in the oil corner is also slightly larger for tricaprln than for triolein which means more ethanol could dissolve in the tricaprln phase. When the storage temperature was elevated to 40 $^{\circ}\text{C}$, the solubility of tricaprln was increased, as expected. The one phase regions seen in Figure 3A for tricaprln at 25 $^{\circ}\text{C}$ was extended to connect the ethanol and the tricaprln corners in one homogenous phase at 40 $^{\circ}\text{C}$ (Figure 3B). Only a small amount of triolein was found in the ethanol solutions phase. It is interesting to note that tricaprln, with higher melting temperature, is more soluble in ethanol than triolein. This indicates that it is the long chains in triolein which are more lipophilic that contribute to the lower solubility.

Contemplating these results with an emulsion-based hand sanitizer formulation in mind, some observations are in order. The interesting part of the phase diagram for this specific formulation type is in the region with 60-85 wt% ethanol in the continuous phase of the emulsion to confirm disinfectant efficiency, and a triglyceride amount between 5 and 30 wt% providing a moisturizing effect. Having these compositions in mind, both triglycerides would be able to form emulsions as these compositions fall safely within the two-phase region.

3.3 Melting temperature of triglycerides

Thermotropic data on melting of the triglyceride fractions extracted from triglyceride-ethanol-water mixtures are shown in Table 2. The melting point, T_{endset} , and enthalpy of melting, ΔH_m for tricaprin decreased proportionally with increasing ethanol concentration. These results can be compared to the ternary phase diagram for tricaprin, which shows that samples with pure ethanol dissolved more tricaprin in the ethanol phase. Thus, it can be hypothesized that pure ethanol is more prone to interact with the lipid phase, affecting the melting properties. For tricaprin, the DSC curve looked similar for both the first and the second scans and there is no reason to suspect polymorphism since T_{onset} for tricaprin corresponds well with the literature value of the β -form at 31.5 °C (50), and ΔH_m for tricaprin ($163.8 \pm 5.6 \text{ J g}^{-1}$) is very similar to the literature value of 168.16 J g^{-1} (50). The melting point, T_{endset} , and enthalpy of melting, ΔH_m for triolein were lower than the literature values (50,51), most probably because of lower purity as our triolein had a purity of 90.2 wt% whereas tricaprin had a purity of 98.5 wt% (51). There was no considerable change in T_{onset} for triolein when mixed with ethanol-water mixture. However, T_{endset} for triolein increased almost 2 °C when the ethanol concentration was increased from 55 to 85 wt% and decreased further for samples mixed with pure ethanol.

Figure 3. Ternary phase diagrams drawn for triglyceride-ethanol-water mixtures. A. Tricaprin-ethanol-water at 25 °C. B. Tricaprin-ethanol-water at 40 °C. C. Triolein-ethanol-water at 25 °C. The red points represent the composition of the prepared triglyceride-ethanol-water samples, and the symbols in other colors represent the composition at the phase boundaries of the hydrophilic phase and triglyceride phase. The upper and lower lines are dashed since the exact curvature of that boundary region is not known.

The high melting point of tricaprins is favorable for topical formulation purposes as it could be used to create a formulation that involves a solid lipid phase which melts upon skin application. However, the higher solubility of tricaprins in ethanol, plus the fact that ethanol could decrease the melting point to some extent should be considered. If the melting point of tricaprins would be lowered, there is a risk that an intended solid formulation starts melting at room temperature or during transport, especially in tropical areas, changing the physical properties of the product.

If other triglycerides than triolein and tricaprins should be considered, the desirable triglyceride should have long chain unsaturated fatty acid residues to decrease solubility and have a melting point not exceeding normal skin temperature.

Table 2. Thermotropic data on melting (T_{onset} , T_{endset} and ΔH_m) of the separated triglyceride fraction in the triglyceride-ethanol-water mixtures at 25 °C for tricaprins (TO) and tricaprins (TC) samples. Tricaprins mixtures stored at different temperatures (25 °C and 40 °C) were handled as replicates of each other. Data for samples with the same ethanol:water ratio but with different triglyceride content was averaged and the corresponding concentration of triglyceride (TG) in the extracted oil fractions, $w_{\text{TG}}(\text{TG})$, is given.

Oil phase of Triglyceride:(Ethanol/Water)	$w_{\text{TG}}(\text{TG})$ (wt%)	T_{onset} (°C)	T_{endset} (°C)	ΔH_m (J/g)
Tricaprins	100	31.9 ± 0.8 (n = 2)	40.0 ± 0.3 (n = 2)	163.8 ± 5.6 (n = 2)
TC:(55/45)	-	25.9 ± 1.9 (n = 6)	36.2 ± 0.9 (n = 6)	134.8 ± 16.1 (n = 5)
TC:(70/30)	94.3 ± 1.4 (n = 3)	25.0 ± 1.5 (n = 6)	36.0 ± 1.8 (n = 6)	141.5 ± 12.5 (n = 6)
TC:(85/15)	90.5 ± 0.6 (n = 3)	24.6 ± 1.7 (n = 5)	34.2 ± 2.3 (n = 5)	149.4 ± 5.7 (n = 5)
TC:(100/0)	84.0 (n = 1)	22.1 ± 0.7 (n = 5)	31.9 ± 3.1 (n = 5)	128.8 ± 12.9 (n = 5)
Triolein	100	-17.3 ± 0.1 (n = 2)	-2.8 ± 0 (n = 2)	60.5 ± 1.2 (n = 2)
TO:55/45 _{avg}	-	-18.6 ± 0.9 (n = 2)	-0.27 ± 0.3 (n = 2)	74.6 ± 1.6 (n = 2)
TO:70/30 _{avg}	95.1 ± 3.0 (n = 3)	-18.0 ± 0.4 (n = 3)	-0.55 ± 0.8 (n = 3)	75.4 ± 1.9 (n = 3)
TO:85/15 _{avg}	97.0 ± 1.5 (n = 3)	-18.0 ± 0.08 (n = 3)	-0.63 ± 1.4 (n = 3)	69.8 ± 1.7 (n = 3)
TO:100/0 _{avg}	93.9 ± 1.0 (n = 3)	-17.8 ± 0.14 (n = 3)	-1.50 ± 1.0 (n = 3)	67.7 ± 0.9 (n = 3)

3.4 Structural properties of starch in ethanol-water solutions

SWAXD data were collected for quinoa starch dispersions in water (starch-H₂O) and in 70 wt% ethanol solution (starch-70%EtOH) at different temperatures. SAXS diffraction data presented in Figure 4A and B reveal the presence of a broad peak at $\sim 0.067 \text{ \AA}^{-1}$ ($d = 94 \text{ \AA}$) and $\sim 0.071 \text{ \AA}^{-1}$ ($d = 89 \text{ \AA}$) for starch-H₂O and starch-70%EtOH separately at 25 °C and 40 °C. This peak has been associated with lamellar ordering of amylopectin side chains (52,53) with alternating crystalline and amorphous lamellae of amylopectin. Furthermore, this peak is present when starch is hydrated and has not been seen in dry starch(54). The lamellar repeat distance has been reported to be 90 Å for many starch species studied (55), but lately it was found that the lamellar repeat distance changes with high starch concentration (52). The present results show that the lamellar structure of quinoa starch at the same concentration (16 wt%) swells less in 70 wt% ethanol solution ($d = 89 \text{ \AA}$), than in a water solution ($d = 94 \text{ \AA}$). Recalculation of the starch concentration based on the water content in the 70 wt% ethanol solution gives a value of 38.8 wt% starch in available water, which is above the eutectic composition ($\sim 35 \text{ wt\%}$ starch) reported by Carlstedt et al. (52) for potato starch. This indicates that the amount of water present has a considerable impact on the structural features of the lamellar structure. The lamellar structure in Pickering emulsions was also probed and it was found to be similar to starch dispersions in water-based emulsions and alcohol-based emulsions respectively (Figure S2, Supplementary data). When the temperature was increased to 60 °C the peak associated with lamellar repeat distance disappeared for starch-H₂O, and water-based Pickering emulsions (Figure S2, Supplementary data), but was still present

at $\sim 0.071 \text{ \AA}^{-1}$ ($d = 88 \text{ \AA}$) for starch-70%EtOH, and alcohol-based Pickering emulsions (Figure S2, Supplementary data), although the intensity was slightly lowered. This can be associated with the loss of crystallinity in the water dispersions when starch gelatinizes and indicates that gelatinization did not occur or is not complete in 70% ethanol solution.

WAXD data for quinoa starch in water and ethanol solutions is presented in Figure 4 C and D at different temperatures. At 25°C , both samples display peaks at $\sim 1.05 \text{ \AA}^{-1}$ ($d = 6.0 \text{ \AA}$), a double peak at $\sim 1.2 \text{ \AA}^{-1}$ and $\sim 1.25 \text{ \AA}^{-1}$ ($d = 5.3$ and $5.0 \text{ \AA}$), and a final peak at $\sim 1.6 \text{ \AA}^{-1}$ ($d = 3.9 \text{ \AA}$). A small peak at $\sim 0.38 \text{ \AA}^{-1}$ ($d = 16.5 \text{ \AA}$), which overlapped with the characteristic peak of the Kapton film used as windows for the sample holder, was detected when mica sheets were used (Figure S3, Supplementary data). This peak is related to the distance between amylopectin side chains within the lamellar structure ("the 100 inter-helix distance") and depends on the presence of moisture (52,53,56). The presence of this peak, in combination with the other peaks present, would suggest that hydrated quinoa starch has a C-type structure in contrast to the crystalline A-type which has been reported before (57–60). One reason could be that previous reports may have measured the starch in a dry state where this peak is not present, or that it was overlooked since it was a very small peak (57). C-type starch is believed to contain a mixture of A- and B-type allomorphs within the granule (61,62). The A-type polymorph crystallizes in an orthogonal unit cell with hexagonal packing and 8 water molecules per unit cell, while the B-type polymorph crystallizes in a hexagonal unit cell with a more open hexagonal packing and 36 water molecules per unit cell (56,63,64).

Figure 4. X-ray diffraction data (A,B,C, and D) and DSC thermograms (E) of quinoa starch dispersions (16 wt%) in water and in ethanol-water solutions (10-50%EtOH). SAXD data displaying starch-H₂O (A) and starch-70%EtOH (B) at 25°C (blue line), 40°C (cyan line) and 60°C (red line). WAXD data displaying starch-H₂O (C) and starch-70%EtOH (D) at 25°C (blue line), 40°C (cyan line) and 60°C (red line). DSC thermograms of quinoa starch dispersions ranging from 0-50 wt% ethanol (E). The data have been offset along the ordinate for clarity.

The crystalline peaks disappear for starch-H₂O upon heating to 60°C but not for starch-70% EtOH in line with SAXS data presented above. This indicates the disruption of the crystalline structure and gelatinization of starch in starch-H₂O, but not in starch-70%EtOH. DSC thermograms of quinoa starch dispersions (16 wt%) in ethanol solutions with increasing ethanol content (wt%) is shown in Figure 4 E and F. Increasing the amount of ethanol up to 50 wt% ethanol (Figure 4E) resulted in shifting of the transition temperatures (T_{onset} , T_p , T_{endset}) to higher temperatures. Above 50 wt% ethanol it was not

possible to determine the transition temperatures as the thermograms fall outside the measurement range (95 °C), and sample evaporation was noticed.

Transition temperatures and enthalpy of gelatinization were plotted against ethanol content in the quinoa starch dispersions (16 wt%) and the calculated water activity of binary ethanol-water solutions in the dispersions (65), Figure 5. In this calculation, we assume starch is not affecting the water activity of the sample, but in reality, it would most likely have a small effect. Apparently, transition temperatures increase with increasing amount of ethanol, or alternatively with lower water activity. $T_{\text{endset}} - T_{\text{onset}}$ was in the same range (10.2-11.6) for all samples, except the 40 wt% ethanol (13.8) and 50 wt% ethanol (18.1) samples.

It is important to recognize if these effects are a result of interactions with ethanol *per se*, or water available for swelling. Water is important for swelling and is also linked to the wetting necessary for mobility and unwinding of amylopectin outer side chains. Since neither the crystallinity nor the structure of starch did change in ethanol solutions, this difference may be connected to the water activity and available water in the dispersions. A similar approach to explain these results has been reported by Evans et al. (66). This feature may be useful from a formulation point of view, in which the higher gelatinization temperatures of starch can be useful for making the product stable and unchanged even during transport and storage in tropical countries.

Figure 5. Transition temperatures: onset temperature, T_{onset} , peak temperature, T_p , and endset temperature T_{endset} and the gelatinization enthalpy plotted against ethanol content in 16 wt% starch dispersions and the water activity in the starch dispersions.

The morphology and surface properties of the starch- H_2O and starch-70%EtOH dispersions were further visualized with SEM, Figure 6. The granules were intact with polygonal shape and particle size of 1-2 μm , and no difference in the morphology was found between starch- H_2O and starch-70%EtOH

starch particles. Starch in starch-H₂O has a higher tendency to aggregate and aggregates attach to each other forming larger cakes. In starch-70%EtOH, on the other hand, aggregation does not occur to the same extent and individual free starch particles can be seen. This observation, in combination with observations made for Pickering emulsions, see Figure 1, suggest that the surface properties of the starch may have been altered by the presence of ethanol. Although ethanol does not seem to alter the crystalline structure of the starch at room temperature, it may have changed the surface properties of the starch. One can imagine that ethanol alters the hydrophobic octenyl succinate substituent in the modified starch (modification by octenyl succinic anhydride (OSA) (67)).

Figure 6. SEM micrographs of dried starch dispersion in water (A-B) and in 70 wt% ethanol (C-D) after spreading on artificial skin (Vitro-Skin®). Starch particles dispersed in water were aggregated together and formed large cakes, whereas starch particles dispersed in 70% ethanol were more loosely aggregated. Scale bars 10 μm (left) and 1 μm (right).

3.5 Structural properties triglycerides in Pickering emulsions

SAXD for Pickering emulsions reproduced the 90 $\text{\AA}$ peak seen for starch dispersions, suggesting that lamellar structure of starch is not disturbed in the emulsions, but still affected by the solvent used. This was observed for both triolein and tricaprln Pickering emulsions.

For triolein Pickering emulsions, a broad peak was found at $\sim 1.36 \text{ \AA}^{-1}$ ($d = 4.60 \text{ \AA}$) corresponding to the fluid hydrocarbon chains. Interestingly, the contribution from the crystalline peaks of starch are not visible in the WAXS region in the presence of triolein (Figure S4, Supplementary data).

The d-spacing for the major Bragg peaks for tricaprln Pickering emulsions are tabulated in Table 3. In addition, some key WAXS patterns are shown in Figure 7 for water-based tricaprln Pickering emulsion (PE TC:H₂O) and alcohol-based tricaprln Pickering emulsion (PE TC:70%EtOH) with the same dispersed:continuous phase ratio of 3:7.

Figure 7. WAXD data of water-based (PE TC:H₂O) and alcohol-based (PE TC:70%EtOH) tricaprins Pickering emulsions with dispersed phase:continuous phase ratio of 3:7. A: PE TC:H₂O after 3 days storage (purple line) and after 1 month storage (blue line) at 25 °C. B: PE TC:H₂O after 3 days storage (orange line) and after 1 month storage (pink line) and after 3 years (dark blue line) at 25 °C. Tricaprin in PE TC:H₂O was not yet crystalline 3 days after preparation, while in PE TC:70%EtOH it had already crystallized.

Tricaprin was crystalline in water-based Pickering emulsions after one month storage, while in alcohol-based Pickering emulsions (70%EtOH) it crystallized in a stable polymorph already within three days, maintained over three years storage at room temperature. Surprisingly, further analysis of X-ray data revealed co-existence of two polymorphs, the metastable β' -form and the most stable β -form, during the whole 3-year time span (Table 3). The difference in crystallization kinetics of tricaprins in water-based contra alcohol-based continuous phase was also surprising. It may be connected to different miscibility of tricaprins with water and ethanol solutions, where presence of small amounts of ethanol in the triglyceride fraction could affect the crystallization kinetics. DSC results (Table 2) of tricaprins mixed with different concentrations of ethanol showed a decrease in T_{onset} with increasing content of ethanol in the aqueous phase. At 55 wt% ethanol solution, the T_{onset} was 25.9 °C in comparison to 25.0 °C for 70 wt% ethanol solution. Further investigations are needed to explain this phenomenon.

3.6 Tactile friction of alcohol-based Pickering emulsions

The tactile friction results do not reveal any difference using either of the two triglycerides in the present formulations with different ethanol-water composition (0 wt%, 55 wt%, and 70 wt% ethanol), Figure S5 (Supplementary Data). Differences in the tactile friction between water- and ethanol-based Pickering emulsions were small, with slightly lower friction for the latter. This difference can be attributed to hydration with water and more extensive starch aggregation in the water-based emulsions contributing to higher friction. Furthermore, it could be that the ethanol evaporates within the first 2.5 minutes during and after application, which means that any effect of different evaporation rates may have not been recorded. A faster evaporation and loss of hydration would result in lower friction coefficients. However, due to the presence of starch particles higher friction relative to the reference substrate was found for all Pickering emulsions after 10 minutes. This also indicates that a residual film was still present and could possibly contribute to the barrier properties of the skin and affect the sensorial perception of the skin.

Table 3. WAXD data of tricaprin and tricaprin Pickering emulsions (PE TC) with dispersed phase:continuous phase ratio of 3:7. The d-spacing (Å) for the major Bragg peaks for tricaprin Pickering emulsion in water (PE TC:H₂O) and in 70 wt% ethanol (PE TC:70%H₂O) at different storage time.

Sample name	Storage time	d-spacings of major Bragg peaks (Å)									
		P ₁	P ₂	P ₃	P ₄	P ₅	P ₆	P ₇	P ₈	P ₉	P ₁₀
β – form Tricaprin *		-	<u>26.7</u>	-	<u>5.38</u>	<u>4.66</u>	<u>4.40</u>	-	<u>3.91</u>	<u>3.81</u>	-
β' – form Tricaprin **		30.6	z	5.48	z	z	z	4.20	z	z	3.62
Tricaprin		30.6	26.7	5.48	5.38	4.66	4.40	4.20	3.91	3.81	3.62
PE TC:H ₂ O (3:7)	3 days	-	-	-	-	4.63(l)	-	-	-	-	-
PE TC:H ₂ O (3:7)	1 month	29.2	26.7	5.48	5.43	4.70	4.47	4.25	3.96	3.86	-
PE TC:70% EtOH (3:7)	3 days		27.9 [#]	5.48	5.43	4.70	4.53	4.23	3.96	3.86*	-
PE TC:70% EtOH (3:7)	1 month	30.6	26.7	5.48	5.43	4.70	4.47	4.28	3.93	3.81	-
PE TC:70% EtOH (3:7)	>3 years	27.9	26.7	5.48	5.43	4.70	4.47	4.20	3.93	3.84	3.64

* β – form of Tricaprin: triclinic subcell P-1, a=5.49 b=12.2 c=27.0 Å, α=86.1 β=87.6 γ=79.8°

** β' – form of Tricaprin: orthorhombic subcell P222, a=5.49 b=12.3 c=30.6 Å, α=β=γ=90.0°

broad peak where double peaks used to show

(l) a broad peak attributed to fluid hydrocarbon chains

The tactile friction of PE TO:70%EtOH (0.5:9.5) and PE TO:70%EtOH (3:7) was compared to two commercial hand disinfectant products, Figure 8, Ethanol Hand Disinfectant and Alcogel. Ethanol Hand Disinfectant is a slightly thickened solution, while Alcogel is a gel. During the application, the normalized friction of the commercial products was high, but was instantly lowered after 2.5 minutes reaching almost reference values. Pickering emulsions, on the other hand, initially showed lower normalized friction, partly as a result of presence of emollient and starch lowering the amount of hydrating liquid. However, the friction was constantly higher than reference values even 10 minutes after application, indicative of a remaining residual film 10 minutes after application.

Figure 8. Normalized friction coefficient measured during spreading of samples between finger and substrate. Commercial products Ethanol Hand Disinfectant (orange bar), Alcogel (purple bar) and Pickering emulsions in 70 wt% ethanol with 5 wt% triolein (blue bar) and 26 wt% triolein (green bar).

The effect of amount of dispersed phase on tactile friction was studied at different triolein concentrations (5-26 wt%), dispersed:continuous phase ratios 5:95 – 30:70, and the results are shown in Figure 9. Despite increasing oil content, the normalized friction coefficient was higher than the reference substrate for all samples during the measurement period. During the application phase, there was a trend in increase of the normalized friction with the amount of oil (and consequently starch content) up to 18 wt% oil, but the friction was initially lower for 26 wt% oil. After 10 minutes the friction was similar for all emulsions, with slightly higher friction for emulsions with 18 and 26 wt% oil. In an earlier study, we have shown that the increased friction coefficients correspond well with a non-greasy afterfeel as a result of the presence of starch particles (43), indicating that the formulations can be prepared with higher oil content without being perceived as greasy.

The effect of amount of dispersed phase on tactile friction was studied at different triolein concentrations (5-26 wt%), dispersed:continuous phase ratios 5:95 – 30:70, and the results are shown in Figure 9. Despite increasing oil content, the normalized friction coefficient was higher than the reference substrate for all samples during the measurement period. During the application phase, there was a trend in increase of the normalized friction with the amount of oil (and consequently starch content) up to 18 wt% oil, but the friction was initially lower for 26 wt% oil. After 10 minutes the friction was similar for all emulsions, with slightly higher friction for emulsions with 18 and 26 wt% oil. In an earlier study, we have shown that the increased friction coefficients correspond well with a non-greasy afterfeel as a result of the presence of starch particles (43), indicating that the formulations can be prepared with higher oil content without being perceived as greasy.

The presence of residual lipid after application would be beneficial in terms of tactile perception, where the consumer experiences the skin as soft and pleasant after the alcohol had killed the germs and evaporated. In addition, the higher friction of the residual film even at 26 wt% triolein suggests that this type of formulation could be perceived as non-greasy despite the high oil content. The results indicate that in terms of tactile friction, the use of Pickering-emulsion-based formulations could be advantageous compared to conventional alcohol-based hand rubs.

Figure 9. Normalized friction coefficient measured during spreading of samples between finger and substrate. Triolein Pickering emulsions in 70 wt% ethanol with different triolein content: 5 wt% (blue bar), 10 wt% (light blue bar), 18 wt% (coral green bar), 26 wt% (green bar).

3.7 Tactile perception of alcohol-based Pickering emulsion by a human sensory panel

In the tactile perception study by a human sensory panel all sensory attributes except ‘moistness’ and ‘odour’ showed statistically significant differences ($p < 0.05$) in the participant rankings, see Table 4. This shows that even for an amateur panel, the assessors could sense significant differences between the samples in this study. The radar chart in Figure 10 is illustrating the rank sums and preference of the formulations for each sensorial attribute.

During the application phase the assessors preferred the samples that were moist, easy to spread, with low cooling effect, easily absorbed or drying quickly, and with smaller amounts of particles (but not necessarily no particles). The commercial product and PE TO:70%EtOH (0.5:9.5) were the two samples most preferred during the application phase. It was further interesting that the assessors could rank the samples in the order of their composition. For instance, ‘degree of particles’ was ranked low for the Ethanol Hand Disinfectant which doesn’t contain starch, and highest for the two Pickering emulsions with high oil and consequently high starch content (12 wt%). In addition, ‘cooling effect’ was ranked high for the samples with highest amount of ethanol (Ethanol Hand Disinfectant and PE TO:70%EtOH (0.5:9.5)), followed by ethanol Pickering emulsion with higher oil content (PE TO:70%EtOH (3:7)), and lastly the water-based Pickering emulsion with higher oil content (PE TO:H₂O (3:7)). Thus, the assessors could also sense the difference in amount of ethanol and dispersed phase in the tested formulations. During the afterfeel phase, the ranking and preference showed larger differences between the formulations. For ‘stickiness’, ‘residual coating’, and ‘greasiness’, the assessors ranked the samples with high oil content (26 wt% triolein) as high. That means that they perceived these samples as more sticky, greasy with more residual coating than the other two samples. More interestingly,

PE TO:70%EtOH (0.5:9.5) could not be distinguished from Ethanol Hand Disinfectant for these attributes suggesting that 5 wt% of triolein was not perceived as more sticky, greasy or to have more residual coating than an emollient-free hand sanitizer. Furthermore, for the perception of ‘moisturizing effect’ there was a significant difference between Pickering emulsions and Ethanol Hand Disinfectant

as a result of incorporating an emollient in these formulations. The sample perceived as softest was PE TO:70%EtOH (0.5:9.5), and it was also the sample that was preferred the most for afterfeel attributes and overall, among the four tested formulations.

Figure 10. Radar charts of rank sums (A) and preference (B) of each formulation for the studied sensory attributes. A. A higher value of rank sums for a certain sensorial attribute means a stronger perceived intensity for this attribute for a given formulation (e.g. most soft). B. Preference of each formulation for a given sensorial attribute. The preference of formulation during the application phase, and the overall preference are also included. Only participants who could perceive attributes marked with (Y/N) ranked the formulations for these attributes and chose their most preferred formulation for these attributes. * Sensory attributes related to the afterfeel phase are star-marked. Formulations were Pickering emulsion with 26 wt% triolein in water (PE TO:H₂O (3:7)) (magenta), and in 70 wt% ethanol (PE TO:70%EtOH (3:7)) (green), and with 5 wt% triolein in 70 wt% (PE TO:70%EtOH (0.5:9.5)) (blue), and compared to commercial Ethanol Hand Disinfectant product (orange).

Table 4. Calculated χ^2 and p-values for each of the attributes from Friedman's test (* for significant results, $p < 0.05$) and rank sums for each of the formulations with multiple comparison using Fisher's least significant difference (LSD), $p < 0.05$). The formulations sharing the same significance group letter have no difference in the ranked perception. Formulations were Pickering emulsion with 26 wt% triolein in water (PE TO:H₂O (3:7)), and in 70 wt% ethanol (PE TO:70%EtOH (3:7)), and with 5 wt% triolein in 70 wt% (PE TO:70%EtOH (0.5:9.5)), and compared to a commercial product, Ethanol Hand Disinfectant.

Attributes	χ^2	p-value	PE TO:H ₂ O (3:7)	PE TO:70%EtOH (3:7)	PE TO:70%EtOH (0.5:9.5)	EHD
Moistness	3.32	0.345	86 ^a	71 ^a	73 ^a	70 ^a
Spreadability	4.84	0.184*	84 ^a	79 ^{ab}	74 ^{ab}	63 ^b
Cooling effect	10.0	0.019*	58 ^a	72 ^{ab}	86 ^b	84 ^b
Amount of particles	-57.84	<0.001*	73 ^a	81 ^a	50 ^b	36 ^b
Absorption	63.64	<0.001*	39 ^a	56 ^a	100 ^b	105 ^b
Stickiness	-83.56	<0.001*	69 ^a	77 ^a	35 ^b	39 ^b
Residual Coating	58.52	<0.001*	101 ^a	103 ^a	50 ^b	46 ^b
Greasiness	58.52	<0.001*	94 ^a	109 ^a	50 ^b	47 ^b
Moisturizing effect	15.56	0.001*	77 ^a	89 ^a	82 ^a	52 ^b
Softness	14.20	0.003*	59 ^a	73 ^a	96 ^b	72 ^a
Odour	-207.8	<0.001*	42 ^a	38 ^a	45 ^a	35 ^a

The words the participants used to describe each formulation by the end of the study were counted and are shown in Figure 11. Both Pickering emulsions with 26 wt% triolein (PE TO:H₂O (3:7) and PE TO:70%EtOH (3:7)) were described as “creamy”, “thick”, “greasy”, as well as “grainy” and “oily”. This indicates that they exhibited a texture that is closer to a cream than a hand sanitizing lotion and that a high oil content would not be preferred for the intended use as highlighted by the rank data for these samples in Figure 10. For the Pickering emulsion with lower oil content, PE TO:70%EtOH (0.5:9.5), the most frequent words used were “cooling”, “thin”, “soft”, “non-greasy” “quick drying”, and “light”. These words are some of the positive characteristics found for alcohol-based hand rubs perceived by focus groups as reported by Greenaway et al. (16). The Ethanol Hand Disinfectant was described as “thin”, “watery”, “quick drying”, “quickly absorb”, and “cooling”. It is clear that there were similarities in the perception of PE TO:70%EtOH (0.5:9.5) and the Ethanol Hand Disinfectant despite the presence of triolein in the Pickering emulsion.

Figure 11. Word clouds of the words used to describe each formulation. Difference in frequency of used word is highlighted by colors and font size (larger size for higher frequency). Formulations were Pickering emulsion with 26 wt% triolein in water (PE TO:H₂O (3:7)), and in 70 wt% ethanol (PE TO:70%EtOH (3:7)), and with 5 wt% triolein in 70 wt% (PE TO:70%EtOH (0.5:9.5)) and compared to commercial Ethanol Hand Disinfectant product.

Furthermore, the questionnaire also revealed some of the preferences for the participants, Figure S6-S7 (Supplementary Data). Most participants preferred a hand sanitizer in either liquid (43%) or gel form (40%). For a moisturizing cream, most participants preferred soft creams (57%) and the rest preferred non-greasy (23%) or thick (17%) creams. When asked to rank important criteria for choosing a possible skin moisturizing antiseptic product, the top 3 criteria, in descending order, were 'Moisturizing effect', 'Disinfectant efficiency' and 'Afterfeel'. Thus, we can conclude that an alcohol-based Pickering emulsion with lower amount of oil can be formulated to fulfill the requirements above, *i.e.*, destroying microbes, moisturizing the skin and adding a pleasant afterfeel. In addition, the flexibility of Pickering emulsions with the possibility to change the ratio between dispersed phase and continuous phase opens the door for new type of Personal Care and Hygiene products with interesting textures.

3.8 Short-term hydration

The results from TEWL measurements for untreated skin sites for all individuals reveal that there was a small decrease in TEWL after 15 minutes, followed by a small increase after 30 minutes, see Figure 12. All the samples showed a slightly lower TEWL after 15 minutes, with no significant difference ($p = 0.05$). After 30 minutes, TEWL decreased a little more for all samples, but two samples were significantly different ($p < 0.05$) to the untreated skin: PE TO:H₂O (3:7) and the Ethanol Hand Disinfectant. The latter contains glycerin while the water-based Pickering emulsion only contains water, starch, and triolein as an emollient. However, it is remarkable that all samples showed an impact on TEWL 30 minutes after one application. It also shows the effect of an occluding film on TEWL even at 5 wt% oil content, and that this effect is similar to the effect of adding glycerin to a traditional hand sanitizer. It can be speculated that a product with a combination of emollient and humectant would have an added value to the skin barrier.

Figure 12. The change in trans epidermal water loss (TEWL) after 15 minutes (left) and 30 minutes (right). One-way Anova with Tukey's Post Hoc test revealed that untreated skin was significantly different from treatments with PE TO:70%EtOH(3:7) and Ethanol Hand Disinfectant after 30 minutes ($p < 0.05$). No significant difference was found between the different treatments after 15 and 30 minutes ($p = 0.05$). Formulations were Pickering emulsion with 26 wt% triolein in water (PE TO:H₂O (3:7)) (magenta), 70 wt% ethanol (PE TO:70%EtOH (3:7)) (green), 5 wt% triolein in 70 wt% (PE TO:70%EtOH (0.5:9.5)) (blue), and Ethanol Hand Disinfectant product (orange).

4. Conclusions

In the present study we have shown that it is possible to create alcohol-based Pickering emulsions using quinoa starch particles as an emulsion stabilizer, and that these formulations are stable for more than three years when stored at ambient conditions. The oil phase should preferentially comprise triglycerides with long chain unsaturated fatty acid residues to minimize miscibility of the polar and the non-polar phase while maintaining a melting point for the triglyceride at or below skin temperature. The quinoa starch was found to have C-type structure, and the gelatinization temperature in ethanol solutions increased with decreasing water content. Ethanol was found to alter the surface properties of the starch granules, which in turn could explain the difference in emulsion properties between alcohol-based and water-based Pickering emulsions. Tactile friction measurements and tactile perception by a human panel concluded that an alcohol-based Pickering emulsion with 5 wt% triolein was the most preferred and ranked highly over a commercial hand sanitizer, specifically for afterfeel attributes.

5. Acknowledgements

We are grateful to the Knowledge foundation (Sweden) for funding the project, and Johan Engblom also thank the Gustaf Th Olsson foundation (Sweden) for financial support. Speximo AB is acknowledged for providing starch particles. We would also like to acknowledge the volunteers for the sensory panel.

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Supplementary Information

Table S1. Phase boundaries in the ternary triglyceride-ethanol-water mixtures of tricaprin and triolein, respectively, determined from visual inspection and thermogravimetric analysis of compositions utilizing the lever rule.

Sample name (TG:EtOH-Water)	Triglyceride (TG) (wt%)	Ethanol (EtOH) (wt%)	Water (wt%)	Storage temperature (°C)	w _{TG} (EtOH) (wt%)		w _{TG} (TG) (wt%)	
					25°C	40°C	25°C	40°C
Triolein (TO)	-	-	-	-	-	-	-	-
TO (5:5) 55% EtOH	50	27.5	22.5	25	-	-	-	-
TO (5:5) 70% EtOH	50	35	15	25	0.05	-	97.66	-
TO (5:5) 85% EtOH	50	41.5	8.5	25	0.24	-	95.06	-
TO (5:5) 100% EtOH	50	50	0	25	2.78	-	94.92	-
TO (3:7) 55% EtOH	30	37.5	31.5	25	-	-	-	-
TO (3:7) 70% EtOH	30	49	21	25	0.07	-	90.91	-
TO (3:7) 85% EtOH	30	59.5	10.5	25	0.38	-	97.14	-
TO (3:7) 100% EtOH	30	70	0	25	3.33	-	92.51	-
TO (7:3) 55% EtOH	70	16.5	13.5	25	-	-	-	-
TO (7:3) 70% EtOH	70	21	9	25	0.06	-	96.79	-
TO (7:3) 85% EtOH	70	25	5	25	0.09	-	98.72	-
TO (7:3) 100% EtOH	70	30	0	25	1.55	-	94.35	-
Tricaprin (TC)	-	-	-	-	-	-	-	-
TC (5:5) 55% EtOH	50	27.5	22.5	25, 40	-	0.05	-	95.05
TC (5:5) 70% EtOH	50	35	15	25, 40	0.24	0.47	95.92	94.01
TC (5:5) 85% EtOH	50	41.5	8.5	25, 40	1.90	2.20	89.89	89.82
TC (5:5) 100% EtOH	50	50	0	25, 40	11.77	1 ϕ	83.99	1 ϕ
TC (3:7) 55% EtOH	30	37.5	31.5	25, 40	-	0.07	-	91.30
TC (3:7) 70% EtOH	30	49	21	25, 40	1.03	0.62	94.37	94.40
TC (3:7) 85% EtOH	30	59.5	10.5	25, 40	2.20	3.97	91.43	89.61
TC (3:7) 100% EtOH	30	70	0	25, 40	-	1 ϕ	-	1 ϕ
TC (7:3) 55% EtOH	70	16.5	13.5	25, 40	-	0.03	-	100
TC (7:3) 70% EtOH	70	21	9	25, 40	0.54	0.21	92.56	99.16
TC (7:3) 85% EtOH	70	25	5	25, 40	1.07	0.97	90.36	94.51
TC (7:3) 100% EtOH	70	30	0	25, 40	-	1 ϕ	-	1 ϕ

Table S2. The attributes evaluated using a ranking test, given in the order they were evaluated in the study with definitions and instructions as specified in the survey.

Attributes,	Definition	Instructions
<i>Application phase</i>		
Moistness	The amount of liquid (wetness) which can be perceived during application.	Spread the sample droplet within the marked circle in a circular motion with 1 finger. (1 circle/second). After 3 circulations, evaluate how moist the samples are.
Cooling effect	The cooling sensation on the skin after application of formulation.	Evaluate how cooled the skin area feels.
Spreadability	The ease to spread the sample over a larger skin area.	Continue distributing the sample on the skin in circular motion (1 circle/second). After another 2 circulations (total 5), evaluate how spreadable the samples are.
Perception of particles	Amount of particles perceived	Continue distributing the sample on the skin in circular motion (1 circle/second). After another 2 circulations (total 7), evaluate how much you perceive the presence of particles.
Absorption	How fast the sample absorbs into your skin.	Continue distributing the sample in a circular motion (1 circle/second). Max 18 additional circulations (total max 30). Evaluate how fast the sample is absorbed by your skin.
<i>Afterfeel phase</i>		
Stickiness	How sticky/gluey the skin feels.	Press your finger withing the marked circle and lift it to evaluate the stickiness. You may repeat it 3 times. Evaluate how Sticky the skin area feels.
Residual Coating	The amount of remaining product on the skin area.	Use your finger to slide softly over the skin in the marked circles and evaluate how much product that remains.
Greasiness	Amount of oil/fat which can be perceived on the skin surface.	Slide your finger within the marked circles and evaluate how greasy/oily the skin area feels.
Moisturizing effect	How moisturized the skin feels.	Let the finger slide slowly in the marked circles and evaluate how soft the area feels.
Softness	How soft and smooth the skin feels.	Let the finger slide slowly in the marked circles and evaluate how soft the area feels.
Odour	How do you perceive the odour of the products	Put the area of your volar forearms where the product was applied closer to your nose to feel the odour from the formulations

Figure S1. Pickering emulsions with different triolein concentration (in rows) and different ethanol-water concentration in the continuous phase. Triolein concentration (dispersed:continuous phase) in descending order from left to right: 41 wt% (5:5), 26 wt% (3:7), 18 wt% (2:8), 10 wt% (1:9), and 5 wt% (0.5:9.5).

Figure S2. SWAXD data of O/W Pickering emulsions comprising 30 wt% triolein and pure triolein sample at different temperature. Samples displayed are freshly made (3 days) triolein emulsions in water (PEm TO), in 70 wt% ethanol (PEtOH TO) and > 3 year sample of 70 wt% ethanol emulsion (PEtOH TO 3yr) in comparison to pure triolein sample. A: SAXD data displaying PEm TO at 25 °C (turquoise blue line) and 60°C (purple line) and triolein (black line) sample. B: WAXD data displaying PEm TO at 25 °C (turquoise blue line) and 60°C (purple line) and triolein (black line) sample. C: SAXD data displaying PEtOH TO at 25 °C (blue line) and 60°C (red line) and triolein (black line) sample. D: WAXD data displaying PEtOH TO at 25 °C (blue line) and 60°C (red line) and triolein (black line) sample. E: SAXD data displaying 3-year-old PEtOH TO sample at 25 °C (blue line) and 60°C (red line) and triolein (black line) sample. F: WAXD data displaying 3-year-old PEtOH TO at 25 °C (blue line) and 60°C (red line) and triolein (black line) sample.

Figure S3. X-ray diffraction data of quinoa starch dispersions (16 wt%) in water (starch-H₂O) and in 70 wt% ethanol (starch-70%EtOH). WAXD data displaying quinoa starchH₂O (**A**) and starch-70%EtOH (**B**) at 25 °C (blue lines), and 60°C (red lines).

Figure S4. WAXD data of water-based Pickering tricaprins emulsions after 3 days (purple line) and 1 month (blue line) at 25 °C (A) and 40 °C (B). WAXD data of 70 wt% ethanol-based Pickering tricaprins emulsions after 3 days (orange line), 1 month (pink line) and after 3 years (dark blue line) at 25 °C (C) and 40 °C (D). WAXD data of 16 wt% starch dispersion in water (aqua green line), in 70wt% ethanol (blue line), pure triolein sample (black line) and of Kapton-film (orange line) at 25 °C (E) and 40 °C (F).

Figure S5. Normalized friction coefficient of tricaprins (A) and trioleins (B) Pickering emulsions with different ethanol-water composition in the continuous phase (0 wt%, 55 wt%, 70 wt% ethanol) with dispersed phase:continuous phase ratio of 7:3. A. Tricaprin Pickering emulsions in water (striped pink bar), 55 wt% ethanol (striped blue bar) and 70 wt% (striped green bar). B. Triolein Pickering emulsions in water (pink bar), 55 wt% ethanol (blue bar) and 70 wt% (green bar).

Figure S6. Preference of moisturizing cream and a type of sanitizer. A. Preference of type of moisturizing cream: Soft (blue), Non-greasy (orange), Thick/Oily (grey), and Thin (yellow). B. Preference of sanitizer dosage form: Liquid (blue), Gel (orange), Spray (grey), and Foam (yellow).

Figure S7. The results of the ranking test when choosing the most important criteria when purchasing a skin moisturizing, antiseptic product.

IV

Will a water gradient in oral mucosa affect transbuccal drug absorption?

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ARTICLE INFO

Keywords:

Buccal drug delivery
Oral mucosa
Permeability
Hydration
Drug transport
Formulation

ABSTRACT

Formulations for buccal drug delivery often comprise polymers to facilitate mucoadhesion based on water sorption. The main objective of the current study was therefore to evaluate the effect of dehydration on drug uptake through oral mucosa. We have used diffusion cells with excised porcine mucosa to study uptake of three alternative drugs (i.e., Metronidazole, Benzylamine and Xylometazoline) together with polyethylene glycol (PEG) as the model polymer for adjusting water activity in the test solutions. Taking drug activity into account, we can conclude that addition of PEG results in a drug flux through mucosa that is about two times lower for Metronidazole and more than 40 times lower for Xylometazoline compared to that from a pure PBS-solution. However, for Benzylamine the uptake through mucosa was more or less the same, which could possibly be due to the high PEG-concentration (65 wt%) affecting the dissociation constant and thus the permeability. These results indicate that an increased water gradient may have the same limiting effect on permeability through oral mucosa as previously seen for skin. Thus, water gradient effects should be a factor to consider when developing buccal adhesive formulations.

1. Introduction

There is a growing interest in using the buccal route both for local delivery of drugs to the oral cavity and for systemic delivery [1–6]. The benefits of delivering drugs via non-keratinized oral mucosa is that the tissue has low enzymatic activity, is highly vascularized and relatively permeable. The hepatic first pass effect can be avoided and the drug can be delivered through sustained controlled release formulations. The buccal route is particularly well suited for drugs with poor oral bioavailability and those in need of fast absorption. One drawback is however the difficulty of retaining the dosage form in contact with the mucosa, preventing displacement and subsequent involuntary swallowing [7,8].

Buccal delivery may be achieved with a number of formulations, many of which are based on mucoadhesive polymers [9,10]. These formulations can come as viscous liquids or gels [11,12], microparticles [13,14] or buccal patches [9,15–17]. The mechanism behind adhesion of buccal patches is not fully understood but water sorption might play a key role as suggested by Borde et al. [18]. For a more thorough description of buccal delivery, the use of mucoadhesive polymers, products under development and corresponding clinical trials and their status, the reader is referred to some recent reviews by Lafleur [19], Montenegro-Nicolini and Morales [20], and Silva et al. [8].

Buccal mucosa is composed of a mucus film covering the non-keratinized stratified epithelium (500–600 µm thick) followed by the basal lamina and finally a connective tissue, comprising the lamina propria and the submucosa [21]. The main barrier to penetration is claimed to be the outermost quarter to one third of the epithelium [21], while the lamina propria, which is rich in blood vessels and capillaries, can function as a sink for drug absorption. Diaz-Del Consuelo et al. [22] observed a significant increase in permeability from extracting the extracellular lipids of pig buccal epithelium. The arrangement of the lipids is however quite amorphous and less organized compared to the lipids in keratinized epithelia, such as the skin barrier, stratum corneum [23,24]. The lipid composition also differs significantly from that of pig skin [22,25] with higher amounts of phospholipids and lower amounts of ceramides and free fatty acids.

To understand how physiological conditions affect molecular transport through biological barriers may have high importance for pharmaceutical product development. In the current study, the effect of water activity (a_w) on drug transport through buccal tissue is investigated. It is known from previous studies on skin that water activity affects the permeability of drugs [26–29]. Even if the physiological conditions in the mouth results in a low water activity gradient in the buccal mucosa, a buccal patch or a concentrated polymer solution could drastically change the local conditions. In fact, properties that have a

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Table 1

Physicochemical data on the investigated APIs; Metronidazole, Benzylamine and Xylometazoline.

Name	Structure	M _w (g/mol)	log D	pKa
Metronidazole		171.15	0.75 [34] −0.02 [34] (pH 7.4)	2.6 [34]
Benzylamine		309.41	1.45 [39] (pH 7) 2.08 [39] (pH 7.6)	9.2 [39]
Xylometazoline		244.38	2.34 [41] (pH 7.4)	10.6 [41]

strong effect on water activity, such as charge, degree of hydrophilicity, swelling capacity etc., are often listed as prerequisites for polymers used in buccal drug delivery [10]. Sparr and Engblom have during the past decades studied transport resistance in lipid layers, such as the ones in stratum corneum, and found that it can be strongly affected by a change in ambient water activity [26,30,31]. They show that an increase in humidity may cause a substantial increase in drug permeability. Sparr and co-workers [32,33] have also described a theoretical model for how a gradient in osmotic pressure can influence the transport properties over a stack of two-component lipid bilayers. The gradient drives water transport and affects both swelling of the bilayers and phase transitions which in turn will affect the diffusion properties of the layer [33]. These findings are probably of a general character and it is thus of interest to see if this also can affect the transport through buccal mucosa.

In this study, we have chosen to work with three active pharmaceutical ingredients (APIs); Metronidazole, Benzylamine and Xylometazoline (Table 1). Metronidazole is an antibioticum primarily used for treatment of anaerobic bacteria and protozoa. Metronidazole is uncharged at physiological pH and has a log P of 0.75 [34]. Lately there have been some articles on Metronidazole for local treatment of infectious diseases in the oral cavity [35–37]. Benzylamine is used as locally acting nonsteroidal anti-inflammatory agent, anaesthetic and analgesic. One key area of use is for treatment of oral ulcers using buccal patches [12] or other buccal delivery systems [38]. Benzylamine is a base which is partly dissociated at physiological pH and with a variability in log D between 1.45 (pH 7) to 2.08 (pH 7.6) [39]. Xylometazoline is primarily used for treating high fever, irritation and congestion of the nasal mucosa induced by cold or allergies, but has also lately been seen to have effects on Rosacea [40]. It was added to this study as we are currently using it in another work concerning nasal delivery. Xylometazoline is a base and thus charged at physiological pH with a log D at pH 7.4 of 2.34 [41].

To summarize, the main objective of the current study was to evaluate a potential effect of the ambient water activity on drug uptake through oral mucosa. This aspect may be of significant importance to pharmaceutical products performance as the desired mucoadhesion often rely on use of various polymers [19,42] which inevitably lowers the water activity of the formulation per se and then dehydrates the tissue on application [43]. To reach this objective we have included three alternative drugs with slightly different properties; Metronidazole, Benzylamine HCl and Xylometazoline HCl (see Table 1) together with polyethylene glycol (PEG1500) for adjusting water activity in the test formulations. Drug absorption was studied *in vitro* using diffusion cells with excised porcine mucosa as membranes. Both buccal and esophageal tissue were used, justified by previous reports from Diaz Del Consuelo and co-workers claiming that they have similar barrier properties [44,45] and the fact that esophagus is larger and thus provides for a higher number of membranes from one individual.

Nonetheless, a validation of the set-up comparing drug penetration through esophagus and buccal mucosa and potential effects of freezing time was included to assure that this does not affect the results. Silicone membranes were used as reference in parallel to porcine mucosa for the permeation studies as the properties of silicone are considered to be inert to changes in ambient humidity and that any differences in flux therefore can be attributed to properties of the individual formulations [26].

2. Materials and methods

2.1. Materials

Metronidazole, Benzylamine HCl and polyethylene glycol 1500 were purchased from Sigma-Aldrich (Stockholm, Sweden). Xylometazoline HCl was received from IPOCHEM, Poland. Sodium chloride, sodium hydrogen phosphate and potassium dihydrogen phosphate (all p.a. reagents) were obtained from Merck (Darmstadt, Germany). Methanol and octanol (both Chromasolv) were obtained from Sigma-Aldrich (Stockholm, Sweden).

2.2. Preparation of membranes

Mucosa was obtained fresh from Lund University, as offal after surgical practise. Therefore, no additional ethical permit was required. Full thickness buccal mucosa was prepared by trimming away adipose tissue and removing underlying muscles. The esophagus was opened longitudinally with a pair of scissors and then trimmed like the buccal mucosa. Further slicing of the mucosa to defined thickness using a dermatome was not applicable. The tissue was used fresh or stored as received at −80 °C until use. Frozen tissue was thawed in PBS before use.

As the major objective of the current study was to evaluate how a change in the water gradient over mucosa affects its permeability, synthetic silicone sheeting (SMI: 0.05" NRV M/M 40D 12" × 12" and Dow Corning: 75 µm, DC Reference 7–4107), which do not respond to a change in ambient water activity [26], were adopted as inert reference membranes.

2.3. Preparation of formulations

The test formulations comprised either of the drugs dissolved in phosphate buffered saline, PBS (130.9 mM NaCl, 5.1 mM Na₂HPO₄·2H₂O and 1.5 mM KH₂PO₄, pH 7.4). In some of the formulations the water activity was adjusted with PEG1500, which however also affects the drug solubility and thereby the activity of the drug. This is important to take into account especially at high drug concentrations closer to its solubility limit where the drug activity often has a significant influence on the release rate from the formulation. Table 3 shows the composition of the different formulations and their corresponding water activity.

2.4. In vitro diffusion studies

Diffusion experiments with Metronidazole and Benzylamine were conducted on flow through cells (PermeGear Inc. USA; orifice 0.64 cm²) at 37 °C, with both silicone sheeting and mucosa as membranes. The flow rate of receptor media (PBS pH 7.4) was set to 1.6 ml/h and aliquots were collected at 1, 2, 3, 4, 6, 8, 12 and 24 h. Franz diffusion cells (PermeGear Inc. USA; V_R = 6 ml, orifice 0.64 cm²) were used to evaluate Xylometazoline transport over mucosa for 24 h, also at 37 °C. Samples were collected (1 ml aliquots) every 2 h during the day, with no sampling overnight. The receptor media was degassed with nitrogen gas for 10 min to avoid air bubbles and tempered before use. Experiments were initiated by adding 1 ml formulation to the donor compartment, which was then sealed with parafilm to avoid

evaporation. Previous studies with excised skin have shown no significant difference in drug permeability from using Franz cells or flow through cells [46,47]. Therefore, it is fair to assume that the drug flux resulting from mucosal response to specific formulations will not be affected by the use of different cells.

2.5. Analytical procedure

Samples from diffusion studies with silicone sheeting as membrane were analysed by spectrophotometry ($\lambda = 319$ nm for Metronidazole and $\lambda = 305$ nm for Benzylamine (Xylometazoline did not penetrate silicone sheeting)), while samples from studies comprising mucosa were analysed by HPLC-UV ($\lambda = 319$ nm for Metronidazole and Benzylamine, and $\lambda = 225$ nm for Xylometazoline). Metronidazole, Benzylamine and Xylometazoline concentrations were calculated from calibration curves of standard solutions prepared in PBS (0.5–30 $\mu\text{g}/\text{ml}$, $R^2 = 0.98$, 2–50 $\mu\text{g}/\text{ml}$, $R^2 = 0.99$ and 5–200 $\mu\text{g}/\text{ml}$, $R^2 = 1.00$, respectively). Metronidazole and Benzylamine were analysed on a Merck Hitachi system with a Phenomenex Gemini C18 column (100 Å, 100×4.6 mm) using methanol and phosphate buffer (10 mM KH_2PO_4) (20:80 v/v) as the mobile phase. The retention time was 1.9 min for Metronidazole and 1.6 min for Benzylamine, using a flow-rate of 2.0 ml/min for the mobile phase and a sample injection volume of 10 μl . Xylometazoline was analysed on a Varian 9012 (Agilent Technologies, USA) HPLC instrument equipped with a Zorbax SB-C8 column of dimensions 4.6×150 mm, 5 μm (Agilent Technologies, USA). The retention time was 4.7 min using a mobile phase consisting of acetonitrile (CH_3CN) - water (35:65, v/v) at a flow rate of 1.0 ml/min and an injection volume of 100 μl in flushed loop. All analyses were performed at room temperature.

The water activity of individual test formulations was determined on a water activity instrument (AQUA LAB Series 3TE, Decagon Devices Inc., Washington). The temperature was set to 37 °C and the instrument was calibrated with 0.5 M KCl. Measurements were made in triplicates with 2–5 min intervals.

2.6. Statistical analysis

The data in the figures are given as mean values with error bars representing standard deviation for replicates at each time point. Statistical outliers were excluded based on a two sided Grubbs' test at $P = 0.05$. Statistical significant difference in permeability between buccal and esophagus tissue and the effect of storage time was calculated according to a Student's t-test using a heteroscedastic two-tailed distribution ($P < 0.05$).

3. Result and discussion

Porcine buccal mucosa resembles both structure and permeability characteristics of human mucosa, which makes it appropriate for use in *in vitro* permeation studies [48]. Pig esophageal mucosa furthermore provides a good model for buccal mucosa due to similar histology and lipid content [22,44,45]. It also comprises a larger area that is easier to excise for *in vitro* experiments compared to the buccal mucosa.

To assure that the experimental set-up gave valid results, potential variations in *in vitro* drug permeability due to type of tissue and effect of storage time were evaluated with Metronidazole as the model drug, (Table 2). Based on Student's t-test we conclude no significant difference in permeability between buccal and esophagus tissue and no visible effect of storage time up to three months at -80 °C prior to conducting the experiment, which is in line with what Nicolazzo et al. observed for storage at -20 °C [49]. Based on these findings and the limited availability of tissue, the experiments in this study were conducted with membranes from both esophagus and buccal mucosa. It should furthermore be mentioned that we have not had an ambition to investigate active transport of the drugs as the time from harvesting

Table 2

Potential effects on Metronidazole permeability in mucosa, depending on type of tissue (buccal or esophageal mucosa) and tissue storage time at -80 °C. The donor formulation comprised 0.5 wt% Metronidazole in PBS, pH 7.4. Steady state flux was determined over the interval 6–12 h, and presented as mean $\pm$ standard deviation.

Tissue	Storage time weeks at -80 °C	J_{ss} ($\mu\text{g}/\text{cm}^2\text{h}$)	n	Student t-test
Buccal		60 ± 24	22	*
Esophagus		56 ± 28	21	0.64
Buccal & Esophagus	0	44 ± 17	6	0.09
	1	61 ± 23	16	*
	4	67 ± 32	11	0.55
	12	52 ± 25	10	0.38

The * indicates the reference data set for student t-test measurements.

tissue to start of the experiment is too long in the case of non-frozen tissue to claim that we are looking at viable tissue.

Representative permeation profiles of Metronidazole through buccal, esophagus and silicone membranes are shown in Fig. 1a. While the drug permeation over silicone membrane is essentially linear with time from start, mucosa generates a lag time of approximately 4 h. The steady state flux of Metronidazole is therefore calculated from data obtained from the linear region between 6 and 12 h. Student t-test did not reveal any significant difference in flux over buccal vs. esophagus tissue (Table 2).

The steady state flux of Metronidazole dissolved in PBS (0.5 wt%) through oral mucosa was around 60 $\mu\text{g}/\text{cm}^2\text{h}$, Table 2. This is more than five times higher than the flux we have observed through excised skin in previous studies [26 and Engblom et al. (unpublished)]. Even though the outer layer of mucosa and skin both consist of a stratified squamous epithelium, the stratum corneum is keratinized and much denser and dryer than mucosa [50]. Thus, it is not surprising that the penetration of a reasonably hydrophilic molecule ($\log P = 0.75$ [34]) would be faster through mucosa than skin. The results are also in line with the higher water penetration through buccal mucosa compared to skin that has been observed by other authors [51,52].

To investigate the potential effect of dehydration on drug uptake through mucosa we have used a simple system where we keep the activity (i.e. chemical potential) of Metronidazole in the donor solutions constant, while the gradient in water activity over the tissue is varied [26]. The water gradient was controlled by addition of increasing amounts of a water soluble polymer (PEG1500) in the donor solution [53,54], while pure PBS served as receptor solution in all experiments. The benefit of a constant drug activity in this experiment is that the different formulations will have the same drug release rate. Any effect seen on drug permeation through the mucosa is then related to changes in membrane properties induced by hydration, which is understood from the generalized Fick's first law of diffusion [55]

$$J_i = -\frac{D_i}{RT} c_i \frac{\partial \mu_i}{\partial z} = -D_i c_i \frac{\partial \ln a_i}{\partial z} \quad (1)$$

where J ($\mu\text{g cm}^{-2} \text{h}^{-1}$) is the flux of a molecule, $D(z)$ ($\text{cm}^2 \text{h}^{-1}$) is the diffusion coefficient at position z (cm), $c(z)$ ($\mu\text{g cm}^{-3}$) is the concentration of the diffusing molecule at position z , and μ and a are the chemical potential and the activity, respectively, of the diffusing molecule.

Since addition of PEG affects the solubility of Metronidazole and thus its activity in the solution, we have matched the drug concentrations to the solubility in the formulations according to the principles described by Björklund et al. [26]. Silicone membranes, which do not respond to a change in water activity, were used in diffusion experiments to confirm that all test-solutions had the same Metronidazole activity. The steady state flux, J_{ss} , was around 2.6 $\mu\text{g}/\text{cm}^2\text{h}$ (Table 3) and the relative flux versus that from Metronidazole dissolved in pure

Fig. 1. Metronidazole permeability through mucosa and silicone membrane compared to literature data on skin. **a** Representative permeation profiles from 0.5 wt% Metronidazole dissolved in pure PBS ($a_w = 0.996$) through buccal and esophagus mucosa (red square and triangle, respectively, left y-axis) and silicone membranes (blue circles, right y-axis). **b** Adjustment of Metronidazole concentration in formulations comprising increasing amounts of PEG1500 ($0.83 < a_w < 0.99$) to achieve fixed drug activity. The relative flux over silicone membrane vs. that from Metronidazole dissolved in pure PBS ($a_w = 0.996$) is shown as confirmation together with corresponding data from Björklund et al. [26] (filled and open diamonds, respectively). **c** Effect of temperature (32 vs 37 °C) on water activities resulting from the specific amounts of PEG1500 added to our test formulations. **d** The relative flux over mucosa of Metronidazole in formulations comprising increasing amounts of PEG1500 ($0.83 < a_w < 0.99$) vs. that from Metronidazole dissolved in pure PBS ($a_w = 0.996$) is shown together with corresponding data from Björklund et al. [26] on skin (filled and open diamonds, respectively). A decrease in water activity has a similar effect on flux over mucosa and skin. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

PBS ($a_w = 0.996$) is plotted in Fig. 1b, together with corresponding data from Björklund et al. [26]. The water activity at 37 °C was also measured for these formulations (Table 3). There are some minor discrepancies between the water activities we obtained at 37 °C and the ones reported by Björklund [26] at 32 °C (Fig. 1c). This is most probably due to the temperature difference, 32 vs. 37 °C.

The effect of dehydration (i.e. an increase in the water gradient over the membrane) on Metronidazole uptake through excised mucosa is shown in Table 3. When lowering the water activity of the donor solution from 1 to below 0.96, the steady state flux decreases from about 60 to 30 $\mu\text{g cm}^{-2} \text{h}^{-1}$. In Fig. 1d our results on mucosa are compared to the data retrieved from Björklund et al. [26] for skin, plotted as the relative steady state flux vs. that from Metronidazole dissolved in pure PBS ($a_w = 0.996$). The flux decay occurs at very similar water activity gradients for both oral mucosa and skin. The 2 times decrease in flux for mucosa is however less pronounced than what has been seen for skin where the decrease is around 10 times [26]. The differences between mucosa and skin can probably be related to the tightness of the stratum

corneum compared to the stratified cells in buccal mucosa and especially the difference in keratinization. The difference in response to a changing water activity gradient might also reflect the difference in lipid composition and phase composition discussed in the introduction.

To verify that the effect of dehydration we see for Metronidazole uptake through mucosa is a more general phenomenon and not specifically linked to one drug, we introduced two other drugs; Benzydamine HCl and Xylometazoline HCl (Table 1). From a parallel study with Metronidazole and skin we acknowledge that flux scales with both the drug concentration and the water gradient as predicted by Ficks law (Eq. (1)) [Engblom et al. (unpublished)]. Hence, the subsequent verification could be done with a fix concentration of the respective drugs without accounting for the inevitable difference in drug activity in the two vehicles as long as solubilities are known. Benzydamine HCl is very soluble in water according to the British Pharmacopoeia [53] but since there are products on the market comprising 0.5 wt% (e.g. Tantum Rosa (Angelini Pharma)) we decided to use the same drug concentration as with Metronidazole in PBS (0.5 wt%). Van de Donk et al. [56] report a

Fig. 2. Effect of changing the water activity gradient on Benzylamine and Xylometazoline permeability through mucosa. **a** Cumulative amount over time obtained from donor formulations comprising 0.5 wt% Benzylamine HCl in PBS (filled diamonds) and 0.5 wt% in the 65 wt% PEG1500 solution (crosses). **b** Cumulative amount over time obtained from donor formulations comprising 2.0 wt% Xylometazoline HCl in PBS (filled diamonds) and 2.0 wt% in the 65 wt% PEG1500 solution (crosses). The dotted lines are included as guidance for the eye and correspond to steady state flux (c.f. Table 3) calculated over the linear part of the curves, 1–3 h and 2–24 h, respectively.

finite water solubility of Xylometazoline HCl of 3 wt% and by choosing 2 wt% drug we expect to obtain a reasonably high drug activity in the test solutions (approx. 0.7 in PBS).

Fig. 2 shows the cumulative amount of a) Benzylamine and b) Xylometazoline over time permeated through mucosa from the two test solutions comprising the same amount of the respective drug. Evidently, Benzylamine permeates excised mucosa at similar rates from both vehicles (J_{ss} (1–3 h) = 78 and 97 $\mu\text{g}/\text{cm}^2\text{h}$), while Xylometazoline permeates almost 20 times faster from the pure PBS-solution, compared to the one also comprising PEG (J_{ss} (2–24 h) = 150 and 8.5 $\mu\text{g}/\text{cm}^2\text{h}$, c.f. Table 3).

In Fig. 3a the permeation experiment on Benzylamine have been reproduced with silicone sheeting instead of mucosa as membrane. Cumulative amount over time obtained from donor formulations comprising 0.5 wt% Benzylamine in PBS and 0.5 wt% in the 65 wt% PEG solution. For Benzylamine in the pure PBS-solution the flux is not linear in the whole region. Thus, the linear flux is calculated for the time interval 1–3 h (Table 3). This deviation, combined with the fact that less than 20% of the loaded drug has passed the membrane after

6 h, implies that Benzylamine HCl has a lower water solubility than stated by the British Pharmacopoeia and that the dissolution rate is rather slow. The flux obtained from Benzylamine in pure PBS-solution was nevertheless 15 times higher than from the vehicle also comprising 65 wt% PEG (J_{ss} (1–3 h) = 243 and 16 $\mu\text{g}/\text{cm}^2\text{h}$, c.f. Table 3). As the barrier properties of silicone sheeting do not respond to a change in water gradient over the membrane and the drug concentration in both vehicles are the same, we may conclude from Ficks law (Eq. (1)) that addition of PEG must have a large impact on Benzylamine solubility, and thus its activity, in the vehicle.

Fig. 3b shows a “calibration curve” of Benzylamine flux from PBS buffer over silicone membrane versus concentration. The 0.5 wt% point does not fall on the straight line, which correlates increasing flux to concentration, indicating that 0.5 wt% is above the solubility for Benzylamine in PBS. Using the “calibration curve” in combination with a horizontal line through the 0.5 wt% point, we may estimate the Benzylamine solubility in PBS from the intercept of the two lines to around 0.14 wt% and the corresponding saturation flux over silicone membrane to around 243 $\mu\text{g}/\text{cm}^2\text{h}$. Furthermore, Benzylamine solubility in

Fig. 3. Benzylamine permeability over silicone membrane. **a** The permeation experiment shown in Fig. 2a has been reproduced with silicone sheeting instead of mucosa as membrane. Cumulative amount over time obtained from donor formulations comprising 0.5 wt% Benzylamine HCl in PBS (filled diamonds) and 0.5 wt% in the 65 wt% PEG1500 solution (crosses). The dotted lines are included as guidance for the eye and correspond to steady state flux (c.f. Table 3) calculated over the linear part of the curves, 1–3 h. **b** Steady state flux of Benzylamine HCl over silicone membrane vs drug concentration in donor formulations (i.e. PBS). From the intercept of the two blue dotted lines, the Benzylamine solubility in PBS at 37 °C is estimated to 0.14 wt% and the corresponding saturation flux over the membrane to around 243 $\mu\text{g}/\text{cm}^2\text{h}$ (see also Table 3). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 3

Drug flux over mucosa and silicone membranes, including API solubility and formulations characteristics (i.e., API and PEG1500 concentrations in PBS pH 7.4, and corresponding water activities). Steady state flux was determined over the linear part of the release curve (the ss-region) which differed in time between the individual APIs. Data are presented as mean $\pm$ standard deviation.

API	API conc.	API Solubility		PEG conc.		a_w at 37 °C	J_{ss} mucosa		J_{ss} silicone		ss-region
	(wt%)	(wt%)	n	(wt%)			($\mu\text{g}/\text{cm}^2\text{h}$)	n	($\mu\text{g}/\text{cm}^2\text{h}$)	n	(h)
Metronidazole	0.50	1.17 ± 0.01^a	3	0		0.996	58 ± 26	43	2.7 ± 0.7	8	6–12
Metronidazole	0.59	1.35 ± 0.01^a	3	25		0.987	34 ± 26	5	2.6 ± 0.9	8	6–12
Metronidazole	0.80	1.84 ± 0.15^a	3	45		0.957	30 ± 19	2	2.3 ± 0.6	6	6–12
Metronidazole	1.33	3.05 ± 0.14^a	3	65		0.865	30 ± 10	2	2.7 ± 1.3	9	6–12
Benzydamine	0.50	$(0.14)^b$	–	0		n.m.	78 ± 17	4	243 ± 24	8	1–3
Benzydamine	0.50	$(7.6)^b$	–	65		n.m.	97 ± 18	4	16 ± 1.7	8	1–3
Xylometazoline	2.00	11.8 ± 1.4^c	4	0		n.m.	150 ± 36	6	< LOD	–	2–24
Xylometazoline	2.70	11.8 ± 1.4^c	4	0		n.m.	271 ± 30	7	< LOD	–	2–24
Xylometazoline	2.00	6.6 ± 0.3^c	2	65		n.m.	8.5 ± 6.1	5	< LOD	–	2–24
Xylometazoline	4.50	6.6 ± 0.3^c	2	65		n.m.	16 ± 2.6	6	< LOD	–	2–24

^a Metronidazole solubility at 32 °C, from Björklund et al., 2010 [26].

^b Estimate of Benzydamine solubility at 37 °C, c.f. Fig. 3b.

^c Xylometazoline solubility at 37 °C.

the 65 wt% PEG solution can be estimated from the ratio in drug flux obtained from the two formulations (Fig. 3a) to around 7.6 wt%. With 0.5% Benzydamine the saturation level in the PEG solution would then be less than 0.1, while saturated in PBS (see also Table 3). These results indicate that PEG enhances uptake of Benzydamine through buccal mucosa despite the fact that it also lowers the water activity of the aqueous solution and thereby dehydrates mucosa on application. The fact that the flux for 0.5 wt% Benzydamine in PBS is lower than that for 0.5 wt% in 65 wt% PEG strengthens this conclusion.

There could be several reasons for this result. One is, as shown by Sarveiya et al. [39], that the ionization and transport of Benzydamine through polymeric as well as skin membranes is strongly dependent on pH. This is due to the solubility constant of Benzydamine. In fact, the combination of changes in solubility and permeability seen in their experiments gave a maximum transport at pH 7.6 with large differences within a small pH interval. Furthermore, the high PEG-concentration (65 wt%) will probably affect the dissociation constant and thus the permeability. Benzydamine has also, according to calculations, a low surface tension and one speculation could be that it in itself influences the barrier properties. The log D for Benzydamine is furthermore higher than for Metronidazole (Table 1) and thus one interpretation could be that the higher lipophilicity of Benzydamine influences the results.

As already mentioned above, Xylometazoline (2 wt%) permeates mucosa almost 20 times faster from the pure PBS-solution, compared to the one also comprising 65 wt% PEG (Fig. 2b, Table 3). This difference is also much more pronounced than for Metronidazole. However, Xylometazoline does not permeate silicone sheeting at detectable levels and therefore further experiments with excised mucosa had to be performed to separate the factors governing permeation (i.e. the gradient in drug activity and the diffusivity within the mucosa, c.f. Eq. (1)). We determined the Xylometazoline saturation concentration in PBS comprising 65 wt% PEG to be 6.6 wt% (Table 3) and fixed the drug concentration in this test solution to 4.5 wt% corresponding to the saturation level of the 2 wt% PBS solution, about 0.7 [41]. A second PBS solution with 2.7 wt% Xylometazoline was appended to have two alternative drug concentrations in each vehicle. Later studies showed aqueous solubility of Xylometazoline HCl from literature to be an underestimate and that the solubility is 11.8 wt% in PBS at 37 °C (Table 3). Fig. 4 shows the steady state flux versus drug activity in the respective formulations based on the drug solubilities determined herein, 11.8 and 6.6 wt% respectively. Evidently, a further increase in Xylometazoline concentration of the PBS solution to compensate for the differences in activity would only progress the difference in flux from the two vehicles and thus we did not perform any additional experiments. Based on the slopes of the curves in Fig. 4 ($k = 1100$ and 25, respectively), taking

Fig. 4. Effect on steady state flux of Xylometazoline vs. increasing drug activity from changing the water activity gradient over mucosa. Donor formulations comprise 2.0 and 2.7 wt% Xylometazoline HCl in PBS (filled diamonds) and 2.0 and 4.5 wt% in the 65 wt% PEG1500 solution (crosses), respectively. The dotted lines are included as guidance for the eye and steady state flux is calculated over the linear part of the release curves, 2–24 h (c.f. Fig. 2b and Table 3).

activity into account, we can conclude that addition of PEG results in a flux through mucosa that is more than 40 times lower than from a PBS-solution.

To summarize, the main objective of the current study was to evaluate a potential effect of the ambient water activity on drug uptake through oral mucosa. PEG1500, which was used to increase the water gradient over the membranes, also affected the solubility of all three drugs in the vehicles. Both these factors were expected to alter drug uptake through the tissue and evidently the magnitude of decrease in Metronidazole flux over mucosa we observed has to be due to more than the increase in solubility (i.e. decrease in activity) alone. For Xylometazoline the effect of an increased water gradient on lowering the flux was even more pronounced than for Metronidazole. However, for Benzydamine the uptake through mucosa was more or less the same, despite an apparent lower drug activity and a more dehydrated tissue in case of the PEG formulations.

Other factors may play a role here. The partition coefficient of Xylometazoline at physiological pH (log D (pH 7.4) 2.34) [41] is

significantly higher than for Metronidazole but only somewhat higher than the one for Benzydamine (Table 1). Thus, if log D in general was the cause of the observed difference seen between Metronidazole and Benzydamine, Xylometazoline performance would be closer to that of Benzydamine. Evidently, it is not likely that the result seen from Benzydamine is due to the higher log D. However, log D for Benzydamine is sensitive to pH in the investigated interval and the high PEG-concentration (65 wt%) may affect the dissociation constant and thus the permeability.

The results indicate that the effects of water activity on skin barrier properties seen by Sparr and Wennerström [33] has a more general biological relevance than only for the lipids in the stratum corneum of skin. Although the ambient water activity at other barriers in the body, such as buccal mucosa or the epithelial cells in the GI tract of course is much more constant than the conditions for skin, use of different types of formulations might affect the water activity at the site of application. Thus, water gradient effects should be a factor to be considered when developing mucoadhesive formulations. There are indeed some results indicating that buccal vaccination using controlled release formulations are less effective than liquid vaccines [57]. There could of course be several reasons for these results but reduced penetration over buccal mucosa could not be excluded.

4. Conclusions

The main objective of the current study was to evaluate the potential effect of dehydration on drug uptake through oral mucosa. PEG1500, which was used to increase the water gradient over the membranes, also affected the solubility of all three drugs in the vehicles. Both these factors were expected to alter drug uptake through the tissue. Taking drug activity into account, we can conclude that addition of PEG results in a drug flux through mucosa that is about two times lower for Metronidazole and more than 40 times lower for Xylometazoline compared to that from a pure PBS-solution. However, for Benzydamine the uptake through mucosa was more or less the same, despite an apparent lower drug activity and a more dehydrated tissue with PEG in the formulations. One possible explanation to this observation is that log D for Benzydamine is sensitive to pH in the investigated interval and the high PEG-concentration (65 wt%) may affect the dissociation constant and thus the permeability. It has also been confirmed that esophageal epithelium is a representative tissue for buccal transport studies *in vitro* and that frozen tissue specimens do not change their diffusional behaviour when stored at -80°C for periods of up to twelve weeks.

The results indicate that the effects of water activity previously seen for skin has a more general biological relevance. Although the water activity at mucosal barriers in the body is much higher and more constant than normal ambient conditions for skin, use of different types of adhesive formulations might affect the water activity at the site of application. Thus, water gradient effects should be a factor to consider when developing buccal adhesive formulations.

Declaration of interest

The authors report no conflict of interest.

Acknowledgement

We are grateful to Ms. Shifa Saleem (Malmö University) for technical assistance and to the Gustav Th Ohlsson Foundation (Sweden) for financial support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jddst.2018.10.004>.

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To cite this article: Abdullah Ali, Marie Wahlgren, Birgitta Rembratt-Svensson, Ameena Daftani, Peter Falkman, Per Wollmer & Johan Engblom (2019) Dehydration affects drug transport over nasal mucosa, Drug Delivery, 26:1, 831-840, DOI: [10.1080/10717544.2019.1650848](https://doi.org/10.1080/10717544.2019.1650848)

To link to this article: <https://doi.org/10.1080/10717544.2019.1650848>

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Published online: 10 Aug 2019.

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RESEARCH ARTICLE

Dehydration affects drug transport over nasal mucosa

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ABSTRACT

Formulations for nasal drug delivery often rely on water sorption to adhere to the mucosa, which also causes a higher water gradient over the tissue and subsequent dehydration. The primary aim of this study was therefore to evaluate mucosal response to dehydration and resolve the hypothesis that mucoadhesion achieved through water sorption could also be a constraint for drug absorption via the nasal route. The effect of altering water activity of the vehicle on Xylometazoline HCl and ⁵¹Cr-EDTA uptake was studied separately *ex vivo* using flow through diffusion cells and excised porcine mucosa. We have shown that a modest increase in the water gradient over mucosa induces a substantial decrease in drug uptake for both Xylometazoline HCl and ⁵¹Cr-EDTA. A similar result was obtained when comparing two different vehicles on the market; Nasoferm® (Nordic Drugs, Sweden) and BLOX4® (Bioglan, Sweden). Mucoadhesion based on water sorption can slow down drug uptake in the nasal cavity. However, a clinical study is required to determine whether prolonged duration of the vehicle *in situ* or preventing dehydration of the mucosa is the most important factor for improving bioavailability.

ARTICLE HISTORY

Received 21 May 2019
Revised 24 July 2019
Accepted 29 July 2019

KEYWORDS

Mucoadhesion; nasal drug delivery; dehydration; water activity; drug transport

Introduction

The nasal route of drug delivery offers many advantages such as high absorption, avoidance of the first pass metabolism, rapid onset of effect and the possibility to circumvent the blood–brain barrier. In addition, due to the large surface area of the nose the nasal cavity offers good absorption for low molecular weight lipophilic drugs with bioavailability close to that of the intravenous route (Davis & Illum, 2003). One of the key advantages are that nasal delivery avoids parenteral injections which contributes to good patient-compliance while still in most cases providing higher bioavailability than oral administration (Wadell, 2002; Davis & Illum, 2003; Kumar et al., 2016). Moreover, nasal administration can be used both for local and for systemic drug delivery. Currently, there are numerous drug formulations used for nasal drug delivery for different indications such as analgesia, acute migraine, nasal congestions and infections (Davis & Illum, 2003; Illum, 2003; Ghori et al., 2015).

Some of the limitations encountered with nasal drug delivery is low permeability of polar molecules and large molecular weight peptides and proteins, as well as the mucociliary clearance (MCC). MCC limits transmucosal absorption by renewing the mucus layer lining of the mucosa every 15–21 minutes (Soane et al., 1999; Davis & Illum, 2003; Illum, 2003). The mucus layer can also limit absorption by binding the drug to mucin, the principle

protein in the mucus. Smaller particles pass easily, while larger or charged particles could get trapped in the gel (Jadhav et al., 2007). After passing the mucus layer, the principle mechanisms of drug absorption through the mucosa include transcellular passive diffusion, paracellular passive diffusion and transcytosis by vesicle carriers (Wadell, 2002; Ugwoke et al., 2005; Jadhav et al., 2007).

Nasal formulations are usually used as solutions, gels, or powders. Other formulation types include suspensions, emulsions and microparticle formulations. Solutions are simple and convenient with good patient-compliance and easy administration through, for example, spray pumps (Lee et al., 2000; Upadhyay et al., 2011). They do however often suffer from poor retention characteristics preventing prolonged close contact with the absorption site. One approach to circumvent MCC is to use mucoadhesive polymer-based gels instead, for example self-gelling systems. Gels are highly desirable when comprising bioadhesive polymers that can prolong contact time at the absorption site and improve bioavailability (Smart, 2005; Ugwoke et al., 2005; Duan & Mao, 2010). Powders are less frequently used but have advantages of prolonged contact time with mucosa due to, for example, water sorption and the possibility to formulate preservative-free products. Polymer and powder-based formulations often rely on water sorption and swelling to adhere to the mucosa. Moreover, aqueous polymer formulations often exhibit

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decrease in water activity (Ninni et al., 1999; Björklund et al., 2010), which induces a higher water gradient over the tissue and subsequent dehydration (Mortazavi & Smart, 1993; Pereswetoff-Morath & Morath, 1998; Marshall et al., 2001, 2004). Several studies have indeed reported an increase in nasal drug delivery following use of bioadhesive formulations to prolong the contact time (Björk & Edman, 1990; Pereswetoff-Morath & Morath, 1998; Ugwoke et al., 2005), while others report on lower permeability (Hansen et al., 2015) and build-up of a physical barrier after repeated administration (Callens et al., 2003). Some studies have furthermore focused on studying barrier forming formulations preventing, for example, allergic rhinitis (Josling & Steadman, 2003; Schwetz et al., 2004; Emberlin & Lewis, 2006; Andersson et al., 2008, 2014). However, none of these studies has discussed the transport-barrier response of the mucosa affecting drug permeability, caused by changes in the water gradient across the mucosa subsequent to formulation administration. When a nasal formulation is administered into the nasal cavity, a water gradient across the nasal mucosa will be induced by difference in water chemical potential of the formulation on the outer part of the mucosa, and the inner side of the mucosa where it is constant (physiological conditions). Previous research has shown that an increased water gradient can be detrimental to drug absorption over both skin and oral mucosa (Björklund et al., 2010; Albèr et al., 2013; Ali et al., 2018). Recent studies on pig gastric mucin have also shown how the mobility of small molecules decrease when the water activity in mucin gels is decreased (Runnsjö et al., 2016). It is not farfetched that application of polymer-based formulations to the nasal mucosa may induce a similar response detrimental to drug absorption.

The primary aim of this study was to evaluate mucosal response to changes in water gradient and resolve the hypothesis that mucoadhesion achieved through water sorption could also be a constraint for drug absorption via the nasal route. In other words, we are interested in how the water activity of formulations affects the nasal mucosa and its permeability.

We have investigated the permeability of two hydrophilic substances, Xylometazoline HCl and radiolabeled Chromium-51 ethylene diamine tetraacetate (^{51}Cr -EDTA), *ex vivo* across porcine nasal mucosa in aqueous solutions where the water activity of the vehicle has been controlled using polyethylene glycol 1500 (PEG1500) (Ninni et al., 1999; Björklund et al., 2010). Xylometazoline HCl is a commonly used nasal decongestant, which when administered to nasal mucosa leads to reduction of mucus and liquid production. It is suitable for treating colds, irritation and congestion of the nasal mucosa induced by allergies. ^{51}Cr -EDTA has been identified as an appropriate model drug for studying absorption through nasal epithelium *in vivo*. It has been used in clinical studies focused on the physiology of the nose (Andersson et al., 2011). It is a safe and stable hydrophilic molecule with a similar size ($M_w = 339 \text{ g/mol}$) to Xylometazoline HCl ($M_w = 281 \text{ g/mol}$) and with high recovery in urine (>90%) (Downes & McDonald, 1964; Greiff et al., 1991). ^{51}Cr -EDTA is

included here with a future clinical study in mind and the possibility to provide a platform for evaluating *ex vivo-in vivo* correlation (EVIVC). A clinical study is expected to provide answers related to duration of the formulation in the nasal cavity, drug absorption through nasal mucosa and the effect of changing the gradient in water activity over the mucosa. The advantages of *ex vivo* studies over *in vivo* studies are that they are faster, fewer animals are required, and by avoiding the presence of plasma proteins in the samples more simple analytical procedures can be used (Lee et al., 1997). *In vivo* studies are nevertheless essential for validation of *ex vivo* results. Clinical studies usually use radiolabeled markers and focus mainly on the physiology of the nasal epithelium (Greiff et al., 1993, 1994; Andersson et al., 2008). A clinical study allows testing the drug formulation on human mucosa with pre- and postmucosal factors such as the active MCC, having a cold, and any enzymatic degradation that can take place. Furthermore, as chelating agents have been reported to act as absorption enhancers (Davis & Illum, 2003; Jadhav et al., 2007), it was decided to investigate if addition of $\text{Na}_2\text{-EDTA}$ ($\text{pK}_a = 2.0, 2.7, 6.2, 10.3, M_w = 372 \text{ g/mol}$) (Dawson, 1986) affects absorption of ^{51}Cr -EDTA.

Two commercial products, Nasoferm[®] (Nordic Drugs AB, Sweden) and BLOX4[®] (Bioglan AB, Sweden), were appended to compare different types of vehicle systems with respect to Xylometazoline HCl permeation over mucosa, *ex vivo*. Nasoferm[®] is a nasal decongestant with a water activity close to that of pure water, which comes as a 0.5 or 1 mg/ml Xylometazoline HCl solution in water, also comprising glycerol as humectant, benzalkonium chloride as preservative and a citrate buffer. BLOX4[®] is a nose spray registered as medical device that relieves nasal allergic symptoms caused by pollen and house dust mite allergy (Andersson et al., 2008, 2011). It is a glyceryl monooleate based microemulsion with low water content, which forms a thin protective barrier on the nasal mucosa, claimed to provide immediate and long-lasting (several hours) effect. The long duration *in situ* is most probably due to the ability of monoglycerides, like glyceryl monooleate, to swell and form liquid crystalline phases when in contact with wet mucosa (Nielsen et al., 1998). Water sorption by the formulation will then inevitably also involve dehydration of the mucosa. As BLOX4[®] is an oil-continuous microemulsion, we cannot determine its water activity. However, it behaves as a low water activity formulation when it swells. BLOX4[®] was included in the study to investigate the potential use as a delivery vehicle for Xylometazoline HCl in comparison to Nasoferm[®]. Both formulations contain a range of excipients, which to some extent might influence the drug penetration over mucosa. However, the content of excipients in Nasoferm[®] is most likely too low to have a major effect on water activity.

Materials and methods

Chemicals

$^{51}\text{Chromium edetate}$ (Chromium (^{51}Cr) EDTA[®], 3.7 MBq/ml solution for injection, GE Healthcare Ltd, UK) comprising 0.64 mg/ml ^{51}Cr -EDTA was obtained from the department of

Translational Medicine, Lund University/Skane University Hospital (SUS Malmö). Xylometazoline HCl (log D (pH 7.4) = 2.34, pKa = 10.6, M_w = 281 g/mol) (Golander & DeWitte, 1985), as well as polyethylene glycol 1500 (M_w = 1500 Da), sodium chloride (NaCl), disodium phosphate dihydrate ($\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$), monopotassium phosphate (KH_2PO_4), sodium hydroxide (NaOH), acetonitrile and methanol were all purchased from Sigma-Aldrich (Stockholm, Sweden). Disodium edetate ($\text{Na}_2\text{-EDTA}$) (pKa = 2.0, 2.7, 6.2, 10.3, M_w = 372 g/mol) (Dawson, 1986) was obtained from Merck (Germany). 0.1 wt% Nasoferm® (Nordic Drugs AB, Sweden) was obtained from a local Pharmacy and BLOX4® (Bioglan AB, Sweden) was kindly provided by Bioglan AB. Ultra high quality (UHQ) water, purified at 25 °C by Elgastat UHQ II model UHQ-PS-MK3 (Elga Ltd., High Wycombe, Bucks, U.K.), was used in all in house preparations.

Preparation of test formulations

Xylometazoline HCl (2.7, 4.5 and 8.1 wt%, respectively) was dissolved in phosphate-buffered saline (PBS, pH 7.4), prepared by mixing 130.9 mM NaCl, 5.1 mM $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, 1.5 mM KH_2PO_4 and adjusting pH with NaOH. Xylometazoline HCl (0.1–5 wt%) was also dissolved in BLOX4® and compared to 0.1 wt% Nasoferm® for reference. ^{51}Cr -EDTA solutions were obtained by dissolving Chromium (^{51}Cr) EDTA® (3.2–32 µg/ml) and $\text{Na}_2\text{-EDTA}$ (0–200 mg/ml) in water. PEG1500 was added to decrease the water activity in the test formulations when applicable (65 wt% PEG1500 (aq) corresponds to a_w = 0.826 at T = 32 °C (Björklund et al., 2010)). All test formulations are listed in Tables 1 and 2.

Solubility measurements of drug formulations

The solubility of Xylometazoline HCl was determined by adding excess amount of drug to the formulations. The samples were then sealed and left stirring at 32 °C. After three days, they were filtered through a hydrophilic PTEE 0.45-µm filter and analyzed with HPLC-UV. Drug activity (a_D) was calculated as the ratio between drug concentration in formulation and the drug solubility in the vehicle.

Water activity measurements

The water activity of Nasoferm® formulation in the present study was determined in triplicate with a bench-top water activity meter (LabTouch-aw, Novasina, Switzerland) and the mean value is provided in Table 1. The unit was calibrated with different solutions of standard saturated salts in the water activity range of interest in the present study.

Preparation of porcine nasal mucosa membranes

Fresh porcine noses were obtained from Lund University as offal after surgical practice. Therefore, no additional ethical permit was required. The porcine noses were transferred to the lab immediately and stored in a freezer at –80 °C until use. Fresh samples were stored in refrigerator (<18 h) before tissue preparation. On preparation, the nose was split in two halves between the nostrils, separating the two nasal cavities. Left and right nasal cavity mucosa were handled equally. The mucosa was carefully separated from the underlying tissue using a scalpel and a tweezer. The nasal mucosa

Table 1. Xylometazoline HCl solubility versus degree of saturation (i.e. activity) in two donor formulations; 4.5 wt% Xylometazoline HCl in 65 wt% PEG (aq) gives the same saturation level as 8.1 wt% in PBS.

Vehicle	Water activity	Drug solubility wt%	Drug conc. wt%	Drug activity	Flux, J_{ss} (1–6h) µg/cm ² h (J_{ss} ± 95% CI)
PBS	0.996*	11.9	2.7	0.22	1035 ± 159 (n = 24)
PBS			4.5	0.45	3091 ± 554 (n = 12)
PBS			8.1	0.68	3806 ± 451 (n = 6)
65 wt% PEG in PBS	0.826*	6.6	4.5	0.68	233 ± 97 (n = 7)
Nasoferm®	0.982	–	0.10	–	29.5 ± 9.0 (n = 6) ⁺
BLOX4®	n.a.	–	0.10	–	2.4 ± 1.6 (n = 3) ⁺
BLOX4®	n.a.	–	0.25	–	3.3 ± 0.5 (n = 3)
BLOX4®	n.a.	–	0.50	–	19.8 and 10.6 (n = 2)
BLOX4®	n.a.	–	1.0	–	37.8 ± 2.5 (n = 3)
BLOX4®	n.a.	–	5.0	–	263.0 ± 21.7 (n = 2)

J_{ss} : steady state flux.

* (Björklund et al., 2010)), ⁺Independent sample t-test showed a statistically significant difference between Nasoferm® (n = 6, M = 29.5, SD = 12.3) and BLOX4® flux (n = 3, M = 2.4, SD = 1.7), t (7) = 3.68, p = .008).

Table 2. Test formulations comprising ^{51}Cr -EDTA are given below together with flux data (J_{ss}) from Flow-through diffusion experiments.

Vehicle	Water activity	^{51}Cr -EDTA conc. µg/ml	$\text{Na}_2\text{-EDTA}$ conc. mg/ml	Flux, J_{ss} (1–2h) µg/cm ² h
65 wt% PEG in H ₂ O	0.826 ^a	32	0.1	0.18 ± 0.22 (n = 3)
H ₂ O	1	32	0.1	1.26 ± 0.66 (n = 3)
		32	200.1	1.35 ± 0.50 (n = 3)
		32	100.1	1.83 ± 0.46 (n = 4)
		32	50.1	1.83 ± 0.23 (n = 3)
		16	50.05	0.78 ± 0.01 (n = 2)
		32	10.1	1.53 ± 0.28 (n = 4)
		3.2	10.01	0.21 ± 0.05 (n = 4)

^aNot measured, assumed to be similar to water activity of 65 wt% PEG in PBS in Table 1.

was then placed on a filter paper wetted with PBS-buffer and small membranes ($\varnothing = 22$ mm) were punched for the permeation study. Each membrane was code-marked with reference to its' origin. The permeability experiments themselves were used to control the tissue viability and integrity. This has been reported to be a meaningful method to assess tissue viability as long as transport time was short before freeze storage of tissues (Shojaei, 1998; Nicolazzo et al., 2003). Potential deviation in flux between experiments was used as an indication, and data from a specific cell were disregarded based on two principles; either due to unreasonably high flux indicating damage to the membrane or for less pronounced variations outliers were excluded using Grubbs' test.

Flow-through cell diffusion studies

Diffusion experiments were conducted on flow through cells (PermeGear Inc. USA) (Bronaugh et al., 1986) at 32 °C, with excised nasal mucosa as membranes. The donor and receptor compartments of the diffusion cell are separated by a membrane (0.64 cm²). To avoid air bubbles the receptor media was degassed with nitrogen gas for 10 minutes before use. Before each experiment, the membranes were hydrated by placing them in the diffusion cells with PBS flowing in the receptor compartment for 1 h. Experiments were then initiated by adding formulation comprising Xylometazoline HCl (1 ml) and ⁵¹Cr-EDTA (128 µl), respectively. The donor cells were sealed with parafilm to avoid evaporation of water, the flow rate of receptor media (PBS, pH 7.4) was set to 1.5 ml/h and aliquots were collected every hour during a 6-hour period. Initially, samples were also collected after 30 and 90 minutes.

Analytical methods

Xylometazoline HCl was analyzed at room temperature on a Varian 9012 (Agilent Technologies, USA) HPLC-UV ($\lambda = 225$ nm) instrument equipped with a Synchronis C8 column of dimensions 250 × 3 mm, 5 µm (Thermo Fisher Scientific, USA). Xylometazoline HCl concentrations were calculated from calibration curves of standard solutions in PBS (25–1000 µg/ml, $R^2 = 1.00$, LOD = 10 µg/ml). The retention time was 8 minutes using a mobile phase comprising acetonitrile (CH₃CN) – water (35:65, v/v). The analysis was carried out at a wavelength of 225 nm and the flow rate of the mobile phase was 1 ml/min, and the injection volume was 20 µl. The radioactivity of ⁵¹Cr-EDTA was counted with an automatic gamma counter (1480 Wizard 3).

Experimental considerations

A central part of this study is to study diffusion, while maintaining steady state conditions. This can be fulfilled when the gradients in water and model drug are kept constant by applying excess amounts of donor drug. When sink conditions are fulfilled, the driving force for diffusion will then be proportional to the concentration gradient. This is expressed

in the generalized Fick's first law of diffusion (Evans & Wennerström, 1999):

$$J = - \frac{D(x)}{RT} c(x) \frac{d\mu}{dx} \quad (1)$$

where $D(x)$ (cm² h⁻¹) is the diffusion coefficient at position x (cm), $c(x)$ (µg cm⁻³) is the concentration of the diffusing molecule at position x , and $d\mu$ (J mol⁻¹) is the chemical potential of the diffusing molecule. It is evident from eq. 1 that when steady state condition is reached, we would expect a linear dependence between flux and concentration (Aulton, 2007). Furthermore, drug release is diffusion-controlled (Fickian diffusion) when the fractional amount of drug released, up to 60%, is proportional to the square root of time (Ritger & Peppas, 1987). This is a useful representation for comparing drug delivery from different formulations.

Data from diffusion studies were analyzed from curves from cumulative permeated mass per membrane area as a function of time. The steady state flux, J_{ss} (µg/cm²h), could be calculated from the slope of the linear region of the curve. Data are presented as cumulative amount, as steady state flux of the model drug across the membrane, or as fraction of drug release (%) over time. Another important experimental aspect in this study is the effect of variations in water gradient on the diffusion coefficient. Thus, the gradient in drug chemical potential needs to be constant, while the gradient in water chemical potential is varied through addition of PEG1500 (Björklund et al., 2010). In order to achieve similar chemical potential of Xylometazoline HCl in formulations of PBS and 65% PEG1500 respectively, the concentration was chosen based on the same degree of saturation (drug activity (a_D) = 0.68) concentration of Xylometazoline HCl in each formulation. At concentrations far below the saturation concentration, such as the case for Nasoferm[®] and BLOX4[®], the drug activity is assumed to be equal to the drug concentration (Atkins & De Paula, 2006; Aulton, 2007). The relation between chemical potential and activity can be realized from the following expression (Atkins & De Paula, 2006):

$$\mu_A = \mu_A^* + RT \ln(a_A) \quad (2)$$

where μ_A (J mol⁻¹) is the chemical potential of A, μ_A^* (J mol⁻¹) is the chemical potential of pure A, R (J K⁻¹ mol⁻¹) is the gas constant, T (°C) the temperature, and a_A the activity of A. Thus, in order to reach similar drug chemical potential in two different formulations, the drug activity needs to be considered at high concentration. While for very dilute concentrations, the actual concentration can be used for comparison (Atkins & De Paula, 2006).

Statistical analysis

The data in the figures are given as mean values with error bars representing confidence interval ($p = .05$) for replicates at each time point. Statistical outliers were excluded based on two sided Grubbs' test at $p = .05$. Statistical significance ($p < .05$) was tested using independent samples t-tests, and one-way analysis of variance (ANOVA).

Results and discussion

Verification of *ex vivo* method: Storage time and membrane origin

During the early development of the method, factors that could have an impact on the methodology were investigated with 2.7 wt% Xylometazoline HCl in PBS as test formulation. No major difference in drug permeability could be detected in mucosa retrieved from different pigs, used fresh ($n=7$) or stored one month ($n=7$) and three months ($n=10$) at -80°C before use (Figure 1(A)). This supports studies on buccal mucosa where it was shown that freezing of tissue did not affect drug permeability (Nicolazzo et al., 2003; Diaz del Consuelo et al., 2005). Neither could we detect any difference between mucosa from inner versus outer parts of the snout (Figure 1(A)). The steady-state flux of 2.7 wt% Xylometazoline HCl in PBS ($\text{pH}=7.4$) over 1–6 hours where determined to $1035 \pm 159 \mu\text{g}/\text{cm}^2\text{h}$ ($n=24$) (Figure 1(B), Table 1).

Barrier response to ambient factors

Drug permeability over excised porcine nasal mucosa from Xylometazoline HCl (4.5 wt%) dissolved in two alternative vehicles with different water activity (Table 1) was investigated *ex vivo* in flow through diffusion cells. The results display a short lag time followed by a high and constant flux (Figure 2(A)). The steady-state flux, J_{ss} (1–6 h), obtained from the two vehicles differs by more than an order of magnitude ($J_{ss}=3091$ vs $233 \mu\text{g}/\text{cm}^2\text{h}$; Table 1) where addition of PEG1500 (65 wt% $a_w=0.826$ at $T=32^{\circ}\text{C}$) appeared to be detrimental to drug transport. This is in line with our previous observations with oral mucosa, although the Xylometazoline HCl flux is about an order of magnitude higher through nasal mucosa from both vehicles (Ali et al., 2018).

However, the effect of thermodynamic activity on drug flux over a membrane also has to be taken into account when studying a particular drug in different formulations. The solubility of Xylometazoline HCl changes when adding PEG1500 to a PBS solution. Thus, maintaining equal drug concentration in the two different formulations will result in a difference in the driving force for diffusive transport (Björklund et al., 2010). Therefore, to confirm the hypothesis that the barrier properties of nasal mucosa respond to a change in ambient water activity, in a similar manner as skin and oral mucosa (Björklund et al., 2010; Ali et al., 2018), we adjusted the Xylometazoline HCl concentration to obtain the same drug activity ($a_D=0.68$) in the two vehicles (i.e. 8.1 wt% in PBS and 4.5 wt% in PEG1500-PBS; Table 1). The results shown in Figure 2(B) confirm the difference in drug permeability from the alternative formulations seen in Figure 2(A). The steady-state flux, J_{ss} (1–6 h), obtained from the two vehicles again differs by more than an order of magnitude ($J_{ss}=3806$ vs $233 \mu\text{g}/\text{cm}^2\text{h}$; Table 1). By excluding the effect of different drug chemical potentials and absence of other excipients that may affect the permeability, it can be concluded that the negative effect on uptake of Xylometazoline HCl is due to the difference in water activity between the two formulations. The water activity of the solutions on either side of the tissues determines the water activity in the tissue (Björklund et al., 2010).

Ex vivo model as predecessor for clinical trials

The steady-state flux over excised nasal mucosa of Xylometazoline HCl in PBS increases proportionally with concentration showing that absorption is driven by the drug activity in the formulation (Figure 2(C)). By extrapolating the trend line in Figure 2(C) to the saturation concentration of Xylometazoline HCl (11.9 wt%, $a_w=1$, Table 1), the maximum drug flux from the current formulation can be estimated to $6091 \mu\text{g}/\text{cm}^2\text{h}$. Based on the findings presented in the

Figure 1. Effects on drug permeability through porcine nasal mucosa from local origin and storage at -80°C were studied with donor formulations comprising 2.7 wt% Xylometazoline HCl in PBS. (A) Steady-state flux (1–6 h) across mucosa with respect to site of excision and storage time before use. (B) Cumulative amount of Xylometazoline HCl over time through randomly chosen mucosa membranes ($n=24$). Bars indicate confidence interval, $p=.05$.

Figure 2. Effect of change in concentration and/or water activity gradient on drug permeability through porcine nasal mucosa. (A) Cumulative amount of Xylometazoline HCl over time obtained from two alternative vehicles, PBS (pH 7.4, $a_w = 0.996$, diamonds) and PBS mixed with 65% PEG ($a_w = 0.826$, crosses) (c.f. Table 1) comprising the same drug concentration, 4.5 wt% ($n = 7$ and 12 respectively). (B) Cumulative amount of Xylometazoline HCl over time obtained from the same donor formulations as in (A), comprising the same drug activity, $a_D = 0.68$ ($n = 6$). (C) Effect of increasing the Xylometazoline HCl concentration on drug flux over mucosa with PBS as the vehicle (pH 7.4, $a_w = 0.996$) (c.f. Table 1); the horizontal dashed line indicates the expected drug flux from a saturated solution, $a_D = 1$ ($n = 6-24$). (D) Drug flux plotted against increased of ^{51}Cr -EDTA concentration in water while maintaining a constant ^{51}Cr -EDTA/ Na_2EDTA ratio ($\mu\text{g}/\text{mg}$), ($n = 2-4$). The permeability is depending predominantly on ^{51}Cr -EDTA concentration. Bars indicate confidence interval, $p = .05$.

previous section and the empirical fact that a water solution often proves to be the most effective vehicle for topical delivery ex vivo (Björklund et al., 2010), this flux can tentatively be taken as the target flux for developing any type of nasal formulation comprising Xylometazoline HCl. However, a water solution would most probably not survive long in the nose as it ought to be removed by the mucociliary clearance within about 15-20 minutes (Wadell, 2002; Davis & Illum, 2003).

Clinical trials are of course inevitable for developing effective nasal drug delivery systems, although the more background knowledge that can be gained ex vivo, and through explorative EVIVC studies the better. ^{51}Cr -EDTA is identified as a suitable model drug to measure absorption across the epithelium (replacing in this case Xylometazoline HCl) as it has been used in several studies on physiology of the nasal epithelium before (Greiff et al., 1991, 1993, 1994; Andersson et al., 2011).

As a first step, we evaluated the concentration dependence of ^{51}Cr -EDTA on permeability ex vivo. The steady state flux over excised nasal mucosa of ^{51}Cr -EDTA in water increased proportionally with concentration (Figure 2(D)). The solution of ^{51}Cr -EDTA was diluted from the original stock solution (Chromium (^{51}Cr) EDTA[®]) by 20 times. It is thus fair to assume that ^{51}Cr -EDTA is highly diluted and that at these concentrations the drug activity could be considered equal to the drug concentration and will be the main driving force for permeation.

Another important aspect to verify was of course if uptake of ^{51}Cr -EDTA through mucosa follows the same water gradient dependence as Xylometazoline HCl. Figure 3(A) shows that decreasing the water activity of the formulation by addition of PEG1500 to the water solution while maintaining a fixed concentration of ^{51}Cr -EDTA (32 $\mu\text{g}/\text{ml}$) do indeed result in a lower permeation. The steady state flux of ^{51}Cr -EDTA

Figure 3. Effect of changing the water activity gradient or composition of formulation on ^{51}Cr -EDTA permeability through porcine nasal mucosa using two alternative vehicles, water ($a_w \sim 1$) and 65% PEG in water ($a_w \sim 0.826$) (cf. Table 1). (A) Cumulative amount of ^{51}Cr -EDTA obtained over time from two alternative vehicles, water ($a_w \sim 1$, squares) and 65% PEG in water ($a_w \sim 0.826$, circles) comprising the same drug concentration, $32 \mu\text{g}/\text{mg}$ ($n = 3$) (cf. Table 2). Bars indicate 95% confidence interval, $p = .05$. (B) Fraction of applied ^{51}Cr -EDTA released versus the square root of time from the alternative vehicles in A. Almost all of the supplied ^{51}Cr -EDTA penetrates from the water solution, while less than 20% is absorbed from the PEG-water mixture. (C) Cumulative amount of ^{51}Cr -EDTA obtained over time with increasing $\text{Na}_2\text{-EDTA}$ concentrations from aqueous donor formulations. ^{51}Cr -EDTA/ $\text{Na}_2\text{-EDTA}$ ratios ($\mu\text{g}/\text{mg}$) are shown as 3.2/10 (diamonds), 16/50 (plus signs and black filled circles), 32/10 (circles), 32/50 (bars), 32/100 (squares) and 32/200 (crosses), $n = 2-4$ (cf. Table 2). Lines are appended as guidance for the eye. Significant differences ($p < .05$) between formulations with increasing ^{51}Cr -EDTA concentrations were shown with one-way ANOVA ($p < .05$). (D) Fraction of applied ^{51}Cr -EDTA released from aqueous donor formulation with increased $\text{Na}_2\text{-EDTA}$ concentrations versus the square root of time. The total data set shows that ^{51}Cr -EDTA permeation scales with its concentration in the vehicle suggesting no effect of the added $\text{Na}_2\text{-EDTA}$. There were no significant differences between the curves ($p = 1.00$) measured with one-way ANOVA ($p < .05$). N.B. The bar symbol (32/50) overlaps with the square symbol (32/100) in C and D.

was $1.26 \mu\text{g}/\text{cm}^2\text{h}$ when administered in pure water and $0.18 \mu\text{g}/\text{cm}^2\text{h}$ when supplied in the PEG1500-water mixture (Table 2). Figure 3(B) furthermore shows that while almost all of the supplied ^{51}Cr -EDTA penetrates from the water solution, less than 20% is absorbed from the PEG1500-water mixture.

The present results confirm that a change in the water gradient over nasal mucosa affect the permeability of both Xylometazoline HCl and ^{51}Cr -EDTA in the same way. It means that ^{51}Cr -EDTA ought to be a suitable radiolabeled molecule for studying uptake of hydrophilic molecules through mucosa from alternative vehicles *in vivo*. It furthermore suggests that flow-through diffusion cells can be used for evaluating the potential use of potent pharmaceuticals *ex vivo* and link these results to *in vivo* studies with healthy volunteers using a less harmful probe (^{51}Cr -EDTA).

Presence and potential use of $\text{Na}_2\text{-EDTA}$

The product, Chromium (Cr-51) EDTA[®], used as a source for ^{51}Cr -EDTA in the present study comprised both radiolabeled ^{51}Cr -EDTA and nonlabeled $\text{Na}_2\text{-EDTA}$ in equimolar amounts to secure that all ^{51}Cr is complexed to EDTA. One obvious question is therefore if the presence of the closely related $\text{Na}_2\text{-EDTA}$ affects the permeation of ^{51}Cr -EDTA through the mucosa. Another question that follows is if $\text{Na}_2\text{-EDTA}$ then can be used as absorption enhancer to adjust the permeation rate of ^{51}Cr -EDTA and thereby allow a decrease in the amounts of radiolabeled compound required for conducting *in vivo* trials with healthy volunteers.

The influence of $\text{Na}_2\text{-EDTA}$ as a potential penetration enhancer for ^{51}Cr -EDTA was investigated by increasing the amounts of $\text{Na}_2\text{-EDTA}$ while maintaining a constant ^{51}Cr -

EDTA to Na₂-EDTA ratio, or by adding increasing amounts Na₂-EDTA to a fixed concentration of ⁵¹Cr-EDTA in the aqueous donor solutions (Table 2). Figure 3(C) shows that drug flux increases with increasing concentrations at fixed ratios of ⁵¹Cr-EDTA (3.2–32 µg/ml) and Na₂-EDTA (10–100 mg/ml). All formulations with equal ⁵¹Cr-EDTA concentration (32 µg/ml) created a cluster with similar permeation profiles and a steady-state flux up to 2 hours ranging between 1.26 and 1.83 µg/cm²h despite the fact that the concentration of Na₂-EDTA was not the same (Figure 3(C) and Table 2). Figure 3(C) shows a trend of increase in drug flux increasing concentration. Significant differences ($p < .05$) were found between formulations with different ⁵¹Cr-EDTA concentrations. From the data shown in Figure 3(D), it is furthermore evident that all formulations have similar release kinetics which complies with 1st order diffusion, and that most of the supplied ⁵¹Cr EDTA penetrates the mucosa during 6 h. Statistical analysis based on regression lines for cumulative amount released versus square root of time and one-way ANOVA analysis for significance ($p < .05$) using the slope and standard error of these curves showed that there were no significant differences between the curves ($p = 1.00$).

No major enhancing effect of Na₂-EDTA could be detected for the penetration of ⁵¹Cr-EDTA, which indicates that the product, Chromium (Cr-51) EDTA[®], is a feasible source for the radiolabeled probe required in our future proof of concept study and that the amount of Na₂-EDTA present in the product does not interfere and can be ignored here.

Products for administration of Xylometazoline HCl

Drug diffusion through excised nasal mucosa for two formulations that are expected to differ considerably in how they affect the water gradient over nasal mucosa was investigated. Nasoform[®] 1 mg/ml (comprising an aqueous solution with high water activity, $a_w = 0.982$) and 1 mg/ml Xylometazoline HCl dissolved in BLOX4[®] (comprising of a microemulsion that can absorb water from mucosa) were compared *ex vivo* (Figure 4). The steady-state flux differs by an order of magnitude where Nasoform[®] appears to be the better vehicle ($J_{ss} = 29.5$ and 2.4 µg/cm²h, respectively), and there was a statistically significant difference between Nasoform[®] ($n = 6$, $M = 29.5$, $SD = 12.3$) and BLOX4[®] flux ($n = 3$, $M = 2.4$, $SD = 1.7$), $t(7) = 3.68$, $p = .008$. Noteworthy is however that both formulations are capable of dissolving higher amounts of drug. The microemulsion is capable of dissolving more than 50 mg Xylometazoline HCl per ml, which is reflected by the progressive increase in flux (e.g. 50 mg/ml $\in$ 263 µg/cm²h, Table 1). A drug concentration of 0.1 wt% will then correspond to less than 2% of the maximum solubility. Nasoform[®] is not that different from the PBS-solution used above (maximum solubility = 11.9 wt%), with respect to overall composition (Table 1, Figure 2(A–B)). Hence, a drug concentration of 0.1 wt% would correspond to less than 1% of maximum solubility. As the drug is highly diluted in both formulation, the drug activity could be considered equal to the drug concentration (0.1 wt%), which will be the main driving force for diffusion. The observed difference in flux, can then be related to the gradient in water chemical potential.

Figure 4. Xylometazoline HCl permeability through porcine nasal mucosa from two commercial vehicles, Nasoform[®] (circles, $n = 6$) and BLOX4[®] (triangles, $n = 3$), both comprising 0.1 wt% drug (cf. Table 1). An independent sample *t*-test showed a statistically significant difference between Nasoform[®] ($n = 6$, $M = 29.5$, $SD = 12.3$) and BLOX4[®] flux ($n = 3$, $M = 2.4$, $SD = 1.7$), $t(7) = 3.68$, $p = .008$.

The large difference between the two formulations is then the gradient in water activity over the mucosa, which they induce on application and the residence time in the nose. However, the duration time of Nasoform[®] is most probably hampered by fast mucociliary clearance. The high water sorption capacity of the microemulsion (BLOX4[®]) is expected to strongly favor duration *in situ*, while drug diffusivity over the mucosa may suffer due to dehydration. Whether good mucoadhesion or more hydrated mucosa is the factor that determines which formulation is the most efficacious has to be resolved in a clinical trial.

Conclusions

In this work, we aimed to set up an *ex vivo* diffusion method to evaluate mucosal response to dehydration and resolve the hypothesis that mucoadhesion achieved through water sorption could also be a constraint for drug absorption via the nasal route. We further wanted to investigate whether this method could serve as a preclinical model to evaluate the potential use of potent pharmaceuticals *ex vivo* and link the results to *in vivo* studies on healthy volunteers using a less harmful probe (⁵¹Cr-EDTA).

We have shown that a modest increase in the water gradient over excised porcine nasal mucosa induces a substantial decrease in drug uptake for both Xylometazoline HCl and ⁵¹Cr-EDTA. The same result was obtained when comparing two vehicles on the market comprising Xylometazoline HCl; Nasoform[®] and BLOX4[®].

Mucoadhesion based on water sorption can slow down drug uptake in the nasal cavity. A subsequent clinical study will determine whether prolonged duration of the vehicle *in situ* or preventing dehydration of the mucosa is the most important factor for improving bioavailability. The Chromium (51) EDTA[®] is an acceptable substitute for Xylometazoline HCl in the foreseen study.

Acknowledgements

We are grateful to Ms. Ingela Engbe and Mr. Torbjörn Sund (Bioglan AB) and to Dr. Lars Söderberg (Lund University) for valuable discussions and technical assistance. We acknowledge the Knowledge foundation (Sweden) and the Gustav Th Ohlsson Foundation (Sweden) for financial support.

Disclosure statement

BLOX[®] is a nose spray manufactured by Bioglan AB that relieves nasal allergic symptoms caused by pollen and house dust mite allergy. It is a medical device and does not contain any active pharmaceutical substance. No other potential conflict of interest was reported by the authors.

Funding

This work was supported by the Gustav Th. Ohlssons Fond and the Knowledge Foundation.

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ISBN 978-91-7877-203-2 (print)

ISBN 978-91-7877-204-9 (pdf)

ISSN 1653-5383

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