Influence of Alkanediols as Alternative Preservatives on the Properties of Semisolid Dosage Forms

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Table of Contents

Summary

Many conventional preservatives for dermal formulations have recently fallen into disrepute due to various reasons, in particular when they are used in pediatric preparations. This results in a high demand for alternative components for antimicrobial preservation. The alkanediols 2-methyl-2,4-pentanediol, 1,2-pentanediol, 1,2-hexanediol and 1,2-octanediol studied in this work are substances which may substitute conventional preservatives. Owing to their amphiphilic structure, they exhibit antimicrobial activity. However, this amphiphilicity also entails a high interaction potential of alkanediols with liquid crystalline structures in cream bases. Moreover, alkanediols may influence drug release as well as skin penetration as a further consequence of their amphiphilic characteristics. Therefore, it was the objective of this thesis to investigate the effects of the above-mentioned alkanediols on the properties of four commonly used semisolid formulations for dermal application.

The first part of the work presents results of the analysis of the interfacial activity of the alkanediols. A positive direct correlation was found between the interfacial activity and the chain length of the alkyl residue of the alkanediols. Consequently, the interfacial activity is linked to the amphiphilicity of the alkanediols.

The effects of the alkanediols on the stability and the inner structure of a nonionic hydrophilic cream (NHC) are described in the second part. Furthermore, this part assesses the preservative effect of the studied alkanediols by means of the test for efficacy of antimicrobial preservation according to the European Pharmacopoeia (Ph.Eur.). The incorporation of alkanediols into the mixed crystals of the cream was demonstrated to augment with increasing chain length, resulting in formulations with a slightly reduced consistency. However, the stability of the formulations was not altered by the addition of alkanediols. The test for efficacy of antimicrobial preservation revealed that the antimicrobial properties are also directly related to the chain length, as increasing chain lengths favor the incorporation of the analyzed alkanediols into microbial membranes.

The third part of the present work addresses the alteration of the release of the model drug triamcinolone acetonide (TAA) by the tested alkanediols. The addition of alkanediols was found to affect the release of TAA from different formulations to various extents. Adding alkanediols to a hydrogel formulation resulted in a slightly

increased release rate of the active ingredient with increasing chain length of the added alkanediol. In contrast to this, longer-chained alkanediols reduced the TAA release rate from all tested creams.

Finally, in the last section, studies of the TAA skin penetration and permeation from the various formulations are presented. Further, the modification of the order of the stratum corneum lipids by the alkanediols is evaluated. The results showed that alkanediols generally increased the TAA penetration. The observed effect again strongly depended on the formulation as well as on the alkanediol used. Since only the longer-chained alkanediols led to a significant disorder of the stratum corneum lipids, the penetration-enhancing effect of the shorter-chained alkanediols is likely caused by other factors.

Overall, the results of this work clearly confirm that the studied alkanediols are very well suited as alternative preservatives in dermal preparations when considering their effects on the consistency of the formulation, the drug release and the skin penetration.

Zusammenfassung

Viele herkömmliche Konservierungsmittel für dermale Formulierungen sind aus verschiedenen Gründen in jüngster Zeit in Verruf geraten, insbesondere, wenn diese in pädiatrischen Zubereitungen eingesetzt werden. Infolgedessen steigt die Nachfrage nach Alternativen für die antimikrobielle Konservierung. Die in dieser Arbeit untersuchten Alkandiole 2-Methyl-2,4-pentandiol, 1,2-Pentandiol, 1,2-Hexandiol und 1,2-Octandiol könnten eine derartige Alternative darstellen. Aufgrund ihrer amphiphilen Struktur sind sie antimikrobiell wirksam. Diese Struktur birgt jedoch auch ein hohes Interaktionspotenzial der Alkandiole mit den flüssigkristallinen Strukturen der Cremegrundlagen. Außerdem können Alkandiole aufgrund ihrer amphiphilen Eigenschaften die Freisetzung sowie die Hautpenetration von Wirkstoffen beeinflussen. Das Ziel dieser Arbeit war es daher, die Auswirkungen von Alkandiolen auf die Eigenschaften von vier häufig verwendeten halbfesten Formulierungen zur dermalen Anwendung zu untersuchen.

Im ersten Teil der Arbeit werden die Ergebnisse der Untersuchung der Grenzflächenaktivität der Alkandiole vorgestellt. Dabei wurde ein positiver direkter Zusammenhang zwischen der Grenzflächenaktivität und der Kettenlänge des Alkylrestes der Alkandiole beobachtet. Die Grenzflächenaktivität steht folglich in direktem Zusammenhang mit der Amphiphilie der Alkandiole.

Die Auswirkungen der Alkandiole auf die Stabilität und die innere Struktur einer nichtionischen hydrophilen Creme (NHC) werden im zweiten Teil beschrieben. Darüber hinaus werden in diesem Teil die konservierenden Eigenschaften der untersuchten Alkandiole anhand der Prüfung auf ausreichende antimikrobielle Konservierung gemäß dem Europäischen Arzneibuch (Ph.Eur.) bewertet. Die Einlagerung der Alkandiole in die Mischkristalle der Creme nahm mit steigender Kettenlänge zu, was zu Formulierungen mit einer leicht verringerten Konsistenz, aber unveränderten Stabilität führte. Die Prüfung auf ausreichende antimikrobielle Konservierung zeigte, dass die konservierenden Eigenschaften ebenfalls in direktem Zusammenhang mit der Kettenlänge stehen, da die Einlagerung der Alkandiole in mikrobielle Membranen mit steigender Kettenlänge begünstigt wird.

Der dritte Teil der Arbeit befasst sich mit der Änderung der Freisetzung des Modellarzneistoffs Triamcinolonacetonid (TAA) durch die getesteten Alkandiole. Die

Zugabe von Alkandiolen beeinflusste die Freisetzung von TAA aus den verschiedenen Formulierungen in unterschiedlichem Ausmaß. Der Zusatz von Alkandiolen zu einer Hydrogelformulierung bewirkte eine leicht erhöhte Freisetzungsrate des Wirkstoffs mit zunehmender Kettenlänge des zugesetzten Alkandiols. Im Gegensatz dazu verringerten die längerkettigen Alkandiole die Freisetzungsrate von TAA aus allen untersuchten Cremes.

Die Untersuchung der Hautpenetration und -permeation von TAA aus den verschiedenen Formulierungen sowie die Veränderung der Anordnung der Lipide des Stratum corneums durch die Alkandiole bildet den letzten Teil der Arbeit. Es konnte gezeigt werden, dass Alkandiole die Hautpenetration von TAA grundsätzlich erhöhen. Dabei hängt der beobachtete Effekt wiederum stark von der Formulierung sowie vom verwendeten Alkandiol ab. Da ausschließlich die längerkettigen Alkandiole zu einer signifikanten Störung der Stratum corneum Lipide führten, scheint die penetrationssteigernde Wirkung der kürzerkettigen Alkandiole durch andere Faktoren verursacht zu werden.

Zusammenfassend bestätigen die Ergebnisse dieser Arbeit deutlich, dass die untersuchten Alkandiole unter Berücksichtigung ihrer Auswirkungen auf die Konsistenz der Formulierung, der Wirkstofffreisetzung sowie der Hautpenetration als alternative Konservierungsmittel in dermalen Zubereitungen sehr gut geeignet sind.

List of Publications

Publication 1

Melanie Sigg and Rolf Daniels, Investigations on Alkanediols as Alternative Preservatives in a Nonionic Hydrophilic Cream. Pharmaceutics. 2020. 12(11), 1117. DOI: 10.3390/pharmaceutics12111117

Publication 2

Melanie Sigg and Rolf Daniels, The effect of alkanediols on the release of triamcinolone acetonide from semisolid dosage forms. International Journal of Pharmaceutics. 2021. 605, 120843, DOI: 10.1016/j.ijpharm.2021.120843

Publication 3

Melanie Sigg and Rolf Daniels, Impact of Alkanediols on Stratum Corneum Lipids and Triamcinolone Acetonide Skin Penetration. Pharmaceutics. 2021. 13(9), 1451. DOI: 10.3390/pharmaceutics13091451

Personal Contribution

Publication 1

Investigations on Alkanediols as Alternative Preservatives in a Nonionic Hydrophilic Cream. Pharmaceutics. 2020. 12(11), 1117. DOI: 10.3390/pharmaceutics12111117

Melanie Sigg and Rolf Daniels

Melanie Sigg

Conceptualization, methodology, investigation, data curation, writing - original draft, visualization

Rolf Daniels

Conceptualization, methodology, writing - review and editing, resources, supervision, project administration

Publication 2

The effect of alkanediols on the release of triamcinolone acetonide from semisolid dosage forms. International Journal of Pharmaceutics. 2021, 605, 120843, DOI: 10.1016/j.ijpharm.2021.120843

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Melanie Sigg

Conceptualization, methodology, investigation, data curation, writing - original draft, visualization

Rolf Daniels

Conceptualization, methodology, writing - review and editing, resources, supervision, project administration

List of Poster Presentations

M. Sigg, R. Daniels, Alkanediols as alternative preservatives for dermal formulations, International PhD Student & PostDoc Meeting, German Pharmaceutical Society, Online Conference, March 2021

M. Sigg, R. Daniels, Alkanediols as alternative preservatives for dermal formulations, 12th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, Online Conference, May 2021

Abbreviations

%	Percent
× g	Multiples of gravity
°C	Degree Celsius
Å	Angstrom
ACG	Aqueous Carbomer Gel
AHC	Anionic Hydrophilic Cream
API	Active Pharmaceutical Ingredient
BC	Basic Cream
BSA	Bovine Serum Albumin
CCD	Charge-Coupled Device
CFU	Colony Forming Units
CHMP	Committee for Medicinal Products for Human Use
cm, cm^2	Centimeter, square centimeter
DAB	German Pharmacopeia (Deutsches Arzneibuch)
DAC	German Drug Codex (Deutscher Arzneimittelcodex)
DSC	Differential Scanning Calorimetry
EMA	European Medicines Agency
e.g.	Exempli gratia; for example
etc.	Et cetera; and other similar things
FDA	Food and Drug Administration
g, mg, µg	Gram, milligram, microgram
G’	Storage Modulus
G’’	Loss Modulus
h	Hour
HPLC	High Performance Liquid Chromatography
i.e.	Id est; that is
log	Decadic logarithm
LOQ	Limit of Quantification
m	Meter
min	Minute
mL, µL	Milliliter, microliter
mm, µm, nm	Millimeter, micrometer, nanometer

n	Number of experiments
NHC	Nonionic Hydrophilic Cream
NRF	New German Formulary (Neues Rezeptur-Formularium)
$P_{octanol\text{-}water}$	Partition coefficient between octanol and water
Pa	Pascal
PAMPA	Parallel Artificial Membrane Permeability Assay
PAT	Profile Analysis Tensiometry
Ph. Eur.	European Pharmacopoeia
R^2	Coefficient of determination
rpm	Revolutions per Minute
s	Second
SAXS	Small-Angle X-ray Scattering
SC	Stratum Corneum
SCCS	Scientific Committee on Consumer Safety
SD	Standard Deviation
sin	Sinus
TAA	Triamcinolone Acetonide
UV	Ultraviolet Radiation
v	Volume
w	Weight
λ	Wavelength

1. Introduction

1.1. Semisolid Dosage Forms for Dermal Application

Dermal preparations are among the most frequently prescribed medicines in Germany. Within this category, corticosteroids are most frequently prescribed, with semisolid dosage forms accounting for a large proportion [1].

The European Pharmacopoeia (Ph. Eur.) defines semisolid preparations for cutaneous use as "preparations intended for application to the skin to deliver active substances for local or systemic effect, or to exert an emollient or protective action". The following categories of semisolid preparations for cutaneous use can be differentiated according to the Ph. Eur.: ointments, creams, gels, pastes and poultices. Semisolid preparations are composed of a simple or compound base in which one or more active pharmaceutical ingredients (API) are dissolved or dispersed [2].

In therapy, it is important to consider that the base may affect the activity of the preparation, and thus the penetration and consequently the bioavailability of the API [3–5]. Therefore, the selection of an appropriate base is a decisive factor for the success of both dermal drug therapy, where the active ingredient is intended to act locally in the skin layers, and transdermal drug therapy, where the active ingredient is administered through the skin for systemic absorption.

In the present work, the investigated API, a glucocorticoid, was incorporated into different creams and gels. Hence, the following subchapters will focus on these two categories of preparations in more detail.

1.1.1. Creams

Unlike ointments, which are single-phase, creams consist of both a lipophilic and an aqueous phase, one finely dispersed in the other. Depending on the continuous phase, lipophilic and hydrophilic creams are distinguished [2]. Moreover, amphiphilic creams represent a special transitional state between hydrophilic and lipophilic creams. Amphiphilic creams possess either a bicoherent structure, implying that both the lipophilic and hydrophilic phases are continuous, or a tricoherent structure with an additional coherent matrix of lamellar crystals [6,7].

In the present work, the influence of alkanediols on two cream bases from the German Pharmacopeia (Deutsches Arzneibuch, DAB) [8] and one from the New German

Formulary (Neues Rezeptur-Formularium, NRF) / German Drug Codex (Deutscher Arzneimittel-Codex, DAC) [9] were investigated. In addition to the two hydrophilic creams “nonionic hydrophilic cream DAB” (NHC) and “anionic hydrophilic cream DAB” (AHC), the amphiphilic cream “basic cream DAC” (BC) was selected. These creams are described in more detail below.

Detailed compositions of the creams are outlined in Table 1.

Table 1. Composition (in % w/w) of the compendial creams used in the present work.

AHC		**NHC**		**BC**	
Emulsifying cetostearyl		Polysorbate 60	5.0	Glycerol monostearate 60	4.0
alcohol type A	9.0	Cetostearyl alcohol	10.0	Cetyl alcohol	6.0
Liquid paraffin	10.5	White soft paraffin	25.0	Medium-chain	
White soft paraffin	10.5	Glycerol 85%	10.0	triglycerides	7.5
Purified water	70.0	Purified water	50.0	White soft paraffin	25.5
				Poly(oxyethlene) 20	
				glycerol monostearate	7.0
				Propylene glycol	10.0
				Purified water	40.0

Schemes of the inner structures of AHC and NHC are displayed in Figure 1 and Figure 2, respectively. The colloidal structure of both creams is considered to be very similar. Their major difference is the charge of the emulsifier. In AHC an anionic emulsifier is used in form of emulsifying cetostearyl alcohol, whereas the nonionic emulsifier polysorbate 60 is employed in NHC.

The liquid crystalline gel structure (A) is formed of cetostearyl alcohol together with either the fatty alcohol sulfates, in case of AHC, or with polysorbate 60, in case of NHC. Water can be accommodated and immobilized (B) within these liquid crystal lamellae. The hydrophilic gel phases of both creams are formed of the mixed crystal (A) and the immobilized water (B). The residual fraction of the cetostearyl alcohol represents the lipophilic gel phase (C), as it crystallizes as a hemihydrate. The remaining fraction of the cetostearyl alcohol encloses the dispersed lipophilic phase (E). (D) represents the bulk water phase immobilized within the coherent matrix structure formed by the two gel phases [6,7].

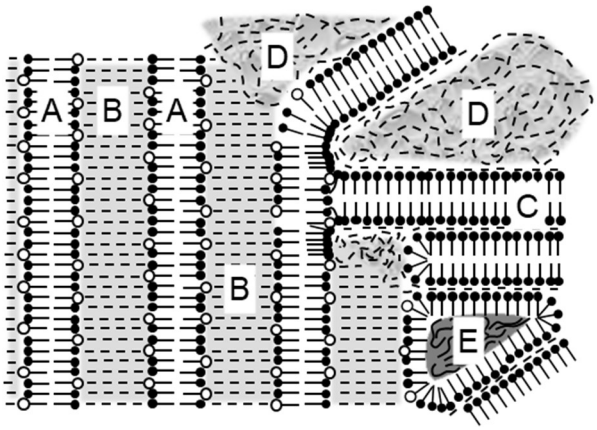

Figure 1. Model structure of the anionic hydrophilic cream DAB, according to Kutz et al. [10]: (A) liquid crystal, lamellar gel structure of emulsifying cetostearyl alcohol, (B) interlamellarly fixed water, (C) lipophilic gel phase of cetostearyl alcohol hemihydrate, (D) bulk water phase, (E) lipophilic dispersed phase.

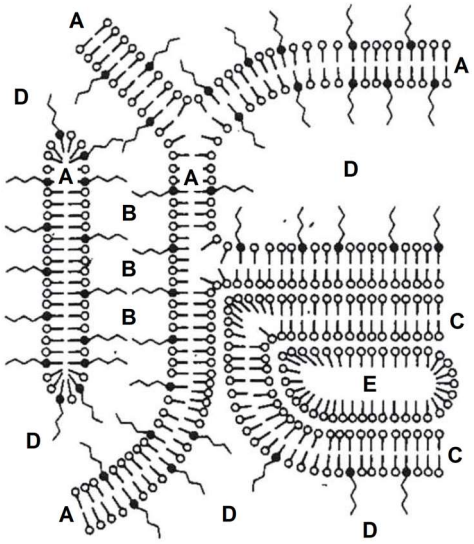

Figure 2. Model structure of the nonionic hydrophilic cream DAB, modified according to Junginger [6]: (A) liquid crystal, lamellar gel structure of polysorbate 60 and cetostearyl alcohol, (B) interlamellarly fixed water, (C) lipophilic gel phase of cetostearyl alcohol hemihydrate, (D) bulk water phase, (E) lipophilic dispersed phase.

Despite their structural similarity, the two creams differ in their compatibilities. Anionic surfactants, like the one contained in AHC, tend to be incompatible with both salts and cationic active ingredients. In addition, skin irritations have been reported in the context of anionic surfactants. Hence, nonionic surfactants are often preferred over anionic surfactants, especially in licensed pharmaceutical preparations [7].

BC, the third cream included in the present study, is an amphiphilic cream whose structure is schematically illustrated in Figure 3. Poly(oxyethlene) 20 glycerol monostearate and cetyl alcohol form mixed crystals in a partly swollen state (A). The fully swollen gel matrix (B) consists of mixed crystals of glycerolmonostearate and cetyl

alcohol in a limited swelling state. The coherent lipophilic phase is denoted with (C) in Figure 3 [6]. Since the matrix structure of the lamellar crystals is also coherent along with the aqueous and lipophilic phases, the BC has not only a bicoherent but a tricoherent structure. Although the matrix is only able to swell to a certain extent, it is fully hydrated even at low water content. This is the reason for the mechanical stability and the good spreadability of the BC [7,10].

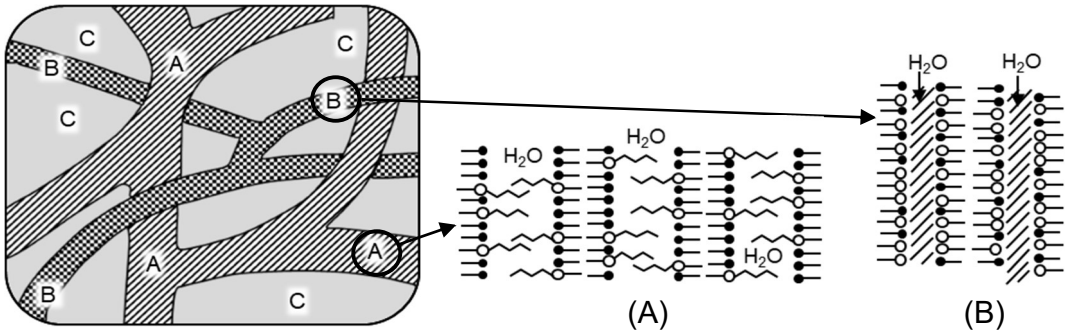

Figure 3. Model structure of the basic cream DAC according to Kutz et al. [10]: (A) Partially swollen gel structure, mixed crystals of poly(oxyethlene) 20 glycerolmonostearate and cetyl alcohol, (B) fully swollen gel matrix, mixed crystals of glycerolmonostearate and cetyl alcohol, (C) coherent lipid phase.

1.1.2. Gels

Gels can be divided into hydrophilic and lipophilic gels, both consisting of a single-phase liquid basis gelled by an appropriate gelling agent [2]. The gelling agent, the solid component, forms a three-dimensional, coherent network which immobilizes the liquid phase [11].

Lipophilic gels (oleogels) are heterogels with a liquid lipophilic phase constituted of hydrocarbons, glycerides, polysiloxanes or wax esters. Typical gel agents for oleogels are zinc or aluminum stearate, colloidal silica or ethyl cellulose [2,12].

The liquid phase of hydrogels, in contrast, consists of water and/or alcohol and may additionally contain a humectant such as propylene glycol or glycerol. The coherent, three-dimensional framework is usually formed by organic macromolecules, like cellulose ethers, carbomers or starch. Rarely, inorganic substances such as bentonite are also employed as gelling agents [2,10]. The most important pharmaceutical gelling agents are carbomers, which are high molecular weight polymers of acrylic acid cross-linked with polyalkene ethers of sugars or polyalcohols. Thus, hydrogels based on carbomers are frequently used in licensed pharmaceutical preparations [7,13,14].

Hence, in the present work, the “aqueous carbomer gel DAB” (ACG) was part of the investigations along with the three creams already described. Its composition is shown in Table 2.

Table 2. Composition (in % w/w) of the compendial ACG used in the present work.

ACG	
Carbomer 50,000	0.5
Sodium hydroxide solution 5%	3.0
Purified water	96.5

In powder form, the carbomer polymer is in a highly coiled state, which is converted to an uncoiled structure when dispersed in water. However, gel formation requires the addition of a base. In the case of ACG, sodium hydroxide is employed for this purpose. The base neutralizes the originally acidic dispersion, and thus deprotonates the acid groups of the polyacrylic acid. The negative charges of the carboxylic acid groups formed along the polymer lead to an electrostatic repulsion. Consequently, the chains are completely stretched, causing strong swelling of the particles and the formation of the gel [14,15].

1.1.3. Vehicles for Dermal Glucocorticoid Treatment

As previously mentioned, the choice of a suitable vehicle is a decisive factor for dermal drug therapy [16]. In the present work, a glucocorticoid was used as an active ingredient incorporated into various formulations. Due to the occlusive effect and the associated increased drug penetration, ointments represent the most suitable choice for the dermal therapy in treating thick, hyperkeratotic and infiltrating skin lesions [4,17,18]. However, the greasy nature poses a challenge for patient compliance [17]. Although creams are less potent compared to ointments as they do not provide an occlusive effect, their acceptance among patients is higher as they have good lubricating properties. As a consequence, the success of the therapy is generally improved. For topical glucocorticoid therapy, creams are usually the formulation of choice for acute and subacute dermatoses [18]. For the treatment of exudative inflammations, hydrogels provide the most suitable base [17] due to their ability of absorbing exudate [19]. Since they are non-greasy and non-occlusive, hydrogels are the most aesthetically elegant formulations [18]. Moreover, another advantage of these products derived from their properties is the possibility of applying them on hairy skin areas [17].

1.2. Preservation of Semisolid Dosage Forms for Cutaneous Application

Antimicrobial preservatives are applied to avoid or inhibit the growth of microorganisms that could pose a risk of contamination or degradation to the product. These microorganisms might proliferate under normal usage conditions. This problem occurs particularly in semisolid formulations that are dispensed as a cream or gel in a multi-dose container for cutaneous application. The aqueous phase of these preparations provides a suitable base for the proliferation of microorganisms [20]. Thus, creams and gels are preparations which are highly susceptible to contamination. Hydrophilic creams, in contrast to lipophilic creams, contain water as the external phase. Therefore, hydrophilic creams are particularly sensitive to microbial contamination. Hence, it is mandatory to protect the aqueous phase of both gels and hydrophilic creams from contamination with microorganisms by adding an appropriate preservative [12]. This allows the shelf life of the formulation to be extended [9]. However, the use of preservatives is not an alternative or even a substitute for hygienic production and good manufacturing practice [20].

The requirements for an ideal preservative are manifold. It should have a bactericidal and fungicidal effect even at low concentrations. In addition, stability and efficacy of the preservative is required over a wide pH range and within a wide temperature interval. Moreover, the preservative should not be toxic in the applied concentration range and should neither be irritating nor sensitizing. Besides, it should be compatible with many active ingredients and excipients as well as packaging materials [21,22].

As indicated above, preservatives are needed in the aqueous phase of the preparations. Therefore, a certain degree of hydrophilicity is required for substances used as preservatives. Simultaneously, on the other hand, a certain extent of lipophilicity is also necessary in order to penetrate the cell wall of the microorganisms, and thus exert the antimicrobial effect. Due to these semi-polar properties, the preservative may partially migrate into the lipophilic phase of the preparation. Consequently, not the overall quantity of the preservative is available for preservation in the hydrophilic phase. As a result, the intended protection against microbial contamination by the preservative may be reduced. Therefore, it is essential to know the partition coefficient $P_{octanol\text{-}water}$ of the preservative [12].

In addition to the partition coefficient, other chemical and physical properties of the substance are relevant for the preservative efficacy as well as its applied concentration. Furthermore, the extent of the initial microbial contamination needs to be known and taken into consideration [20].

Currently, no substance can meet all the above requirements. Consequently, it is necessary to individually decide for each formulation which preservative is suited best.

1.2.1. Classical Preservatives

According to the DAC/NRF, sorbic acid, benzoic acid, and methyl 4-hydroxybenzoate and propyl 4-hydroxybenzoate are considered the most important preservatives used in extemporaneous formulations [23]. Table 3 provides an overview of these preservatives with their maximum and standard concentrations for dermal application.

Table 3. Overview of the most important preservatives for dermal application with their maximum and standard concentrations.

Substance	Maximum concentration	Standard concentration
Sorbic acid and its salts	0.6% (acid) [24]	0.1% [25]
Benzoic acid and its sodium salt	2.5% (acid) rinse-off products [24] 0.5% (acid) leave-on products [24]	0.1-0.2% [26]
Methyl 4-hydroxy-benzoate and its salts Propyl 4-hydroxy-benzoate and its salts	0.4% (acid) [27] 0.14% (acid) [27]	Mostly in mixtures of methyl and propyl ester 0.075% + 0.025% [28]

Sorbic acid or potassium sorbate are the presently preferred preservatives for semisolid formulations due to their good tolerability [23,29]. Additionally, sorbic acid is the only preservative that may be used without restrictions in products for children [30]. Sorbic acid, however, is only effective in the undissociated acid form. For this reason, the use of sorbic acid is only appropriate in formulations that have a pH value lower than 5.5. Because of the low water solubility of sorbic acid itself, potassium sorbate is often used in combination with citric acid.

In addition to sorbic acid, benzoic acid plays a major role in the preservation of extemporaneous preparations [23]. The pKs value of benzoic acid is lower than that of sorbic acid. For this reason, the pH value of the formulation should be below 4.5 to ensure that sufficient quantities of the substance are present in the antimicrobially

active acid form [23,31]. This is often achieved by combining sodium benzoate with an appropriate amount of citric acid.

Parabens are alkyl esters of para-hydroxybenzoic acid. Various esters are used as preservatives, with their combinations playing a particularly important role, as this approach enables the antimicrobial effect to be enhanced [32]. The partition coefficient of parabens rises with increasing chain length of the alkyl residue. Accordingly, parabens with longer-chained alkyl residues can partially distribute into the lipid phase of the formulation. Thus, they are only available to a small extent in the hydrophilic phase, in which they are needed for preservation.

However, the main problem associated with the use of parabens as preservatives is the finding that particularly parabens having a higher molecular weight may act as endocrine disruptors. They interfere with the hormone system, and thus can exert a harmful effect on the human organism. Parabens primarily exhibit an estrogenic effect, which augments with increasing chain length of the alkyl residue [33–36].

Therefore, the use of isopropyl, isobutyl, phenyl, benzyl and pentyl 4-hydroxybenzoate is already prohibited in cosmetic products since 2014 [37]. In addition, the maximum concentration of methyl and ethyl 4-hydroxybenzoate in cosmetics is limited to 0.4% (as acid). Currently, the use of propyl and butyl 4-hydroxybenzoate in cosmetics is still considered to be safe up to a concentration of 0.14% (as acid). The maximum concentration of all parabens contained in a cosmetic product is restricted to ≤ 0.8% [27]. Although the use of a single product preserved with parabens does not generally pose a risk to human health, a significant part of the population uses several products in parallel that each may contain parabens as preservatives. Moreover, such products may be used in excessive quantities. This cumulative application potentially results in exposures exceeding the limit of safety [38].

Unlike for cosmetics, there are currently no restrictions on the use of certain parabens as preservatives for medicinal products for cutaneous use. In practice, methyl and propyl 4-hydroxybenzoate are the main preservatives used in both extemporaneous preparations and proprietary medicinal products [16]. Nevertheless, the Committee for Medicinal Products for Human Use (CHMP) of the European Medicines Agency (EMA) recommends avoiding the use of parabens in medicinal products for children as far as possible [39].

In addition to the endocrine effect, the sensitizing effect of parabens is also frequently discussed [34]. A health warning has to be added to the package leaflet of finished medicinal products containing parabens due to the possible allergenic effect [40]. However, the Scientific Committee on Consumer Safety (SCCS) considers parabens to be safe in the maximum concentrations mentioned above [35]. The German Federal Institute for Risk Assessment (Bundesinstitut für Risikobwertung, BfR) comes to the same conclusion and points to the fact that some other preservatives have a significantly higher allergenic potential than parabens. The sensitization potential of parabens is less than 1%, whereas many other preservatives and ingredients, such as methylisothiazolinone, show considerably higher sensitization rates [34,41].

Over the last years, more and more preservatives have fallen into disrepute, even if their supposed risks are not necessarily objectively justified. Nevertheless, consumers are increasingly concerned and avoid products preserved with conventional preservatives. In addition, some preservatives are severely restricted in their concentrations of use or even banned due to ongoing, constantly evolving risk assessments of the SCCS [42]. In addition, very few preservatives can be used for children, as the percutaneous absorption into children's skin is increased. As a consequence, special care must be taken when adding preservatives to products intended for use on pediatric skin. The various items briefly discussed above result in a growing demand for alternative compounds which can be used for antimicrobial preservation.

1.2.2. Alternative Preservatives

Preservative-free products would be preferred in response to growing consumer demand, based on concerns about conventional preservatives. However, this approach is not applicable with respect to microbial growth in aqueous preparations, especially in multiple dose containers. Otherwise, the shelf life would have to be severely limited, creating an inconvenience for users [9,43].

Yet, a preparation is already deemed to be free of preservation if the preservative is not listed in Annex V of the Regulation No 1223/2009 on cosmetic products [24]. Substances that have an antimicrobial activity but are not listed in this regulation are referred to as alternative preservatives. Accordingly, the primary function of alternative

preservatives in a formulation is not preservation, rather they act as moisturizers, penetration enhancers, solvents, antioxidants, etc. Nevertheless, they additionally protect the product from microbial degradation due to their preservative effect [44].

Since 2017, the “Technical document on cosmetic claims” is available [45]. In 2019, an annex became applicable dealing with the claim "free from". According to this document, no claims should be made with "free from preservatives" if products contain an ingredient that is not listed in Annex V of the Regulation No 1223/2009 but still has a protective effect against microorganisms. This document is not legally binding, and only serves as an interpretation guideline. Therefore, it is still possible to launch products on the market that contain alternative preservatives and declare these products as "preservative-free".

Thus, more and more investigations are focusing on the entire or at least partial substitution of conventional preservatives by alternatives ones having better safety profiles [46–48]. There are numerous substances that can potentially be used for alternative preservation. Some examples are presented in the following.

Many essential oils and extracts of natural origin have very effective antimicrobial properties, and hence are used for alternative preservation. Examples include thyme oil, essential oils of *Rosmarinus officinalis*, *Cinnamomum zeylanicum* and *Calamintha officinalis*. Further, fragrance ingredients of plant origin such as p-anisic acid and levulinic acid are also used as preservatives [43]. Phenylethyl alcohol is also a natural fragrance compound which is used for preservation purposes, both alone as well as in combination with other substances [49]. Its bacteriostatic effect is a result of affecting the permeability of the bacterial cell wall [50].

Ethylhexylglycerin is another frequently used substance for alternative preservation. It is an emollient, a mild humectant and a perfume with antimicrobial properties [51]. The preservative effect of ethylhexylglycerin on its own is not sufficient, but in combination with other alternative or conventional preservatives the effect is very strong, as ethylhexylglycerin enhances the uptake of the other preservatives into the microorganisms [43,52].

Fatty acids and their monoesters such as glyceryl caprylate and glyceryl caprate are emulsifiers, emollients and penetration enhancers for the delivery of active ingredients into the skin [51]. They have an amphiphilic structure and can therefore interact with cell structures of microorganisms, exhibiting an antimicrobial effect [46].

Alkanediols, which are the subject of this work, have a similar structure as the latter class of substances. The most promising alkanediols for alternative preservative applications include 1,2-pentanediol, 1,2-hexanediol, 1,2-octanediol and 2-methyl-2,4-pentanediol (hexylene glycol) [53,54]. Figure 4 presents the chemical structure of these substances. 1,2-Pentanediol, 1,2-hexanediol and 1,2-octanediol are 1,2-alkanediols which have vicinal hydroxyl groups at C1 and C2 positions as can be seen from their chemical structures. They differ only in the number of carbon atoms in their alkyl chain. However, 2-methyl-2,4-pentanediol deviates from this structure, since it has its two hydroxyl groups at positions C2 and C4.

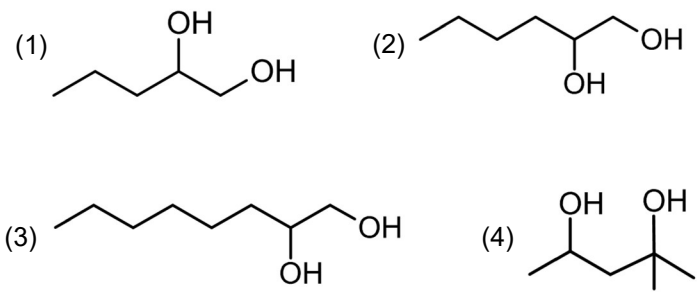

Figure 4. Chemical structures of the alkanediols investigated: (1) 1,2-pentanediol, (2) 1,2-hexanediol, (3) 1,2-octanediol, (4) 2-methyl-2,4-pentanediol.

As a result of the two hydroxyl groups on one side and the lipophilic alkyl residue on the other side, alkanediols possess amphiphilic properties. This peculiarity enables alkanediols to penetrate the lipid bilayer of the cell membranes of microorganisms [55]. This effect results in an inhibition of the substrate transport as well as a leakage of vital cellular components. As the alkyl chain length increases, the antimicrobial effect rises because the incorporation into the cell membranes is improved. The water solubility, on the other hand, diminishes with increasing chain length [56]. As mentioned earlier, a preservative effect can only be achieved if the preservative is at least partially solved in the aqueous phase of the preparation. Thus, the preservative effect of alkanediols having alkyl chain lengths exceeding that of 1,2-octanediol decreases, although the

antimicrobial effect is pronounced [54]. Accordingly, the antimicrobial properties and the preservative effect do not strictly correlate.

In addition to their preservative effect, alkanediols exhibit moisturizing [57], solubilizing [54] and penetration-enhancing properties [58,59]. Therefore, these substances are used as multifunctional excipients in various cosmetic formulations.

In general, the replacement of conventional preservatives with alternative substances does not guarantee the complete elimination of undesirable irritating reactions or sensitization [43,56,60]. Although alkanediols have shown minor sensitizing effects in some studies [56], the Cosmetic Ingredient Review Expert Panel has assessed the use of the alkanediols listed above as safe when applied in the standard concentrations [61,62].

As discussed above, many conventional preservatives are considered as not being safe for use in products for children. This problem cannot be completely resolved with alternative preservation either. Currently, for many substances, the data basis is insufficient for reliably judging their safe usage in products for children. However, the manufacturer of 1,2-hexanediol declares the safety of application in pediatric products for at least this alkanediol [63].

1.3. Skin as an Application Site for Drugs

The human skin, with its very large surface area of about 2 m² [64], plays a major role in both local and systemic drug therapy as well as in the cosmetics industry. Its primary function is to act as a barrier to protect the body from external influences. Moreover, it controls the body temperature as well as the water loss and serves as a permeability barrier in addition to providing a protection against microorganisms and mechanical impacts [65].

1.3.1. Structure of the Human Skin

From the skin surface to the inside, three tissues can be distinguished: the epidermis, the dermis and the hypodermis.

The outermost layer of the epidermis is the stratum corneum (SC), the horny layer, which forms the main entrance barrier into the human body. It contains dead skin cells from the underlying skin layers [66]. These so-called corneocytes are chemically and physically resistant cell remnants of the terminally differentiated keratinocytes found in the viable epidermis. Corneocytes comprise a core composed mainly of keratin surrounded by an envelope. Their cell organelles and cytoplasm have vanished during the cornification process [65]. The structure of the SC can be described by the brick-and-mortar model [67], as illustrated in Figure 5. The corneocytes form the bricks and the lipid fraction forms the mortar, which consists mainly of ceramides, cholesterol, cholesteryl esters and fatty acids [68] in a lamellar order. Additionally, corneocytes are linked by corneodesmosomes, which contribute to the cohesion of the SC. Overall, this skin layer has a total thickness of approximately 10 to 20 µm [65].

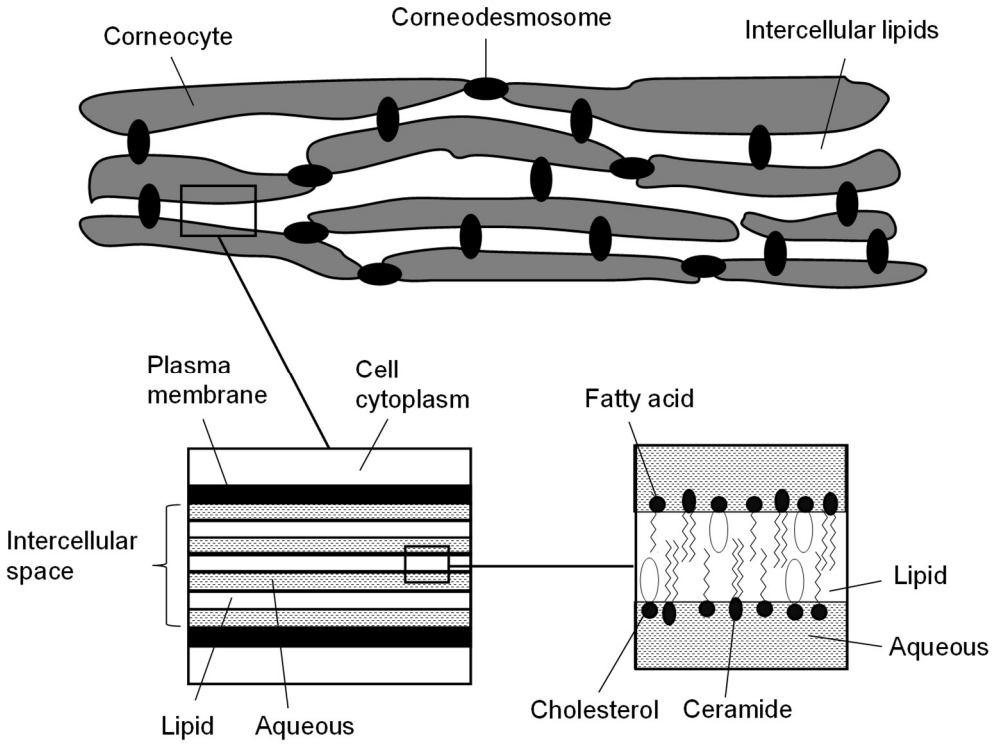

Figure 5. Structure of the stratum corneum modified according to Barry [69] and Harding [70].

Below the SC, the viable part of the epidermis comprises the stratum lucidum (present only in thicker skin of the palms and soles), stratum granulosum, stratum spinosum and stratum basale. The epidermis consists mainly of keratinocytes. Moreover, this skin layer contains Merkel cells, one of the most sensitive touch receptors, Langerhans cells, which are responsible for immune reactions, and melanocytes for skin pigmentation [65,66,71,72].

The epidermis is followed by the dermis, which gives the skin its tensile strength and elasticity [65]. It consists of fibroblasts, mast cells as well as collagenous and elastic fibers, synthetized by fibroblasts, and microfibrils. The filling substance is composed of glycosaminoglycans and proteoglycans. Further, the dermis contains blood vessels that supply the skin and nerves [71].

The hypodermis, which is located beneath the dermis, contains larger blood vessels as well as nerve fibers and fatty tissue. This layer is essential for energy storage and metabolism. In addition, it has endocrine functions, provides thermal insulation and protects the body from injury [65,71].

1.3.2. Dermal Absorption

Penetration into and permeation through the skin is desired to a minor or major extend, depending on the function of the substance applied to the skin. For example, antifungal medicines or sunscreens should only act on the skin. Topical formulations that are intended to have a local effect, such as preparation with glucocorticoids, should locally act in the skin, particularly in the viable parts of the epidermis. However, active ingredients applied transdermally, like hormones, should exert a systemic effect. In the latter, absorption of the API into the blood vessels is required [65].

The dissolution within and the release from the formulation is the first step in drug absorption of active ingredients from dermal formulations. After being released, the active substance distributes into the SC.

Active substances that are intended to act locally in the viable epidermis and dermis or to be absorbed into the systemic circulation need to overcome the highly organized SC which represents the main barrier for dermal penetration. There are several different possibilities to overcome this barrier. Besides the transepidermal route, a route via the pores is also feasible, which can be further subdivided into the transfollicular route through the hair follicles and the transglandular route via the sweat glands. Although hair and sweat glands represent only a small proportion of the total surface area of the skin, it has recently been found that this pathway plays a considerable role in the absorption of active substances through the skin [73–75].

The transepidermal route can be further distinguished between the transcellular and the intercellular route. On the transcellular route, the substance passes directly through the SC, having to overcome both the hydrophilic interior of the keratinocytes and the SC lipids. Since the substance must therefore repeatedly partition from lipophilic to hydrophilic regions, the transcellular route is considered to be unlikely [76]. The intercellular route proceeds through the lipid matrix of the SC and is the preferred penetration path for the majority of agents because the permeate does not have to pass through the corneocytes [73,77]. For hydrophilic substances, a path along the corneodesmosomes is most probable [73,76].

After passage through the SC, actives can distribute into the viable epidermis. Subsequently, the substance diffuses through the viable epidermis and enters the

upper dermis. At this point, the compound can be absorbed by the capillary network, and thus eventually exert a systemic effect [78], which is desired in transdermal administration but unwanted if a local effect is intended.

The absorption of substances into the skin occurs primarily via passive diffusion. For most active substances, the diffusion through the SC is the step which limits the rate of the skin absorption. The steady-state permeation rate J [$kg \cdot m^{-2} \cdot s^{-1}$] of an API through the SC can be described by Equation 1 [58,79–81]

$$J = \frac{D_m \cdot c_{s,m}}{L} \cdot \frac{c_v}{c_{s,v}} \quad (1)$$

wherein D_m [$m^2 \cdot s^{-1}$] stands for the diffusion coefficient of the API in the SC, $c_{s,m}$ [$kg \cdot m^{-3}$] is assigned to the solubility of the API in the SC and L [m] is the thickness of the SC, and thus is the diffusion path length. c_v [$kg \cdot m^{-3}$] describes the API concentration dissolved in the vehicle and $c_{s,v}$ [$kg \cdot m^{-3}$] is the solubility of the API in vehicle. Thus, the ratio $c_v/c_{s,v}$ represents the degree of saturation of the API in the formulation and comprises a range of $0 < c_v/c_{s,v} \leq 1$.

This approach assumes that the SC provides a uniform barrier. Further this description supposes that the transport rate is determined by the diffusion through the skin barrier and that the API is dissolved in the vehicle and has a constant concentration therein.

A number of conditions have to be fulfilled to ensure a dermal or transdermal activity of an active ingredient. Firstly, the drug has to have a molecular weight below 500 Daltons [82]. The active ingredient can only diffuse easily through the SC if this requirement is met. Both diffusion coefficient and the permeation rate decrease with rising molecular weight. Secondly, an adequate lipophilicity is required for distribution and diffusion through the SC. At the same time, the drug needs to be sufficiently soluble in water to allow its distribution into the viable epidermis. To achieve this property, it is beneficial for the $\log(P_{octanol\text{-}water})$ to have a numerical value in the range 1 to 3 [83]. Recent research suggests that active ingredients for dermal administration may have a wider range of the $\log(P_{octanol\text{-}water})$ value than has previously often been assumed, i.e., 1 to 4 [84]. However, if the lipophilicity is too high accumulation in the lipophilic areas of the SC may occur. This accumulation leads to long lag times and the formation of a drug depot in the SC [65,85]. Thirdly, the melting point of the API affects its dermal absorption. Substances with low melting points, below 200 °C [86],

show a high solubility in the SC lipids. For this reason, the diffusion rate into the SC is increased [87]. Moreover, two other parameters are relevant for the dermal absorption of an API: the topological polar surface area, which should be less than or equal to 100 $Å^2$, and the number of aromatic rings, which should be less than or equal to 2 [84].

1.3.3. Skin Penetration Enhancers

Topically applied APIs are often of limited efficacy due to their poor skin penetration, despite meeting all the criteria outlined in the previous chapter. To solve this problem and increase the skin penetration, various techniques have been developed. These include the use of chemical penetration enhancers, vehicle systems like microemulsions, supersaturated formulations or physical approaches such as iontophoresis, electroporation and microneedles [79,88–93]. The last listed physical methods are technically demanding and require a high level of patient compliance, even though much efforts have been made to deploy these techniques as usable tools [94]. The use of chemical penetration enhancers is considerably easier to implement. Since chemical penetration enhancers are one of the subjects of the present work, this approach is discussed in more detail below.

As previously described, skin penetration of substances is a highly complex process. However, three possible ways of increasing skin penetration can be derived based on Equation 1.

The first possibility for enhancing skin penetration is to increase the degree of saturation of the API in the formulation. This is expressed by the ratio $c_v/c_{s,v}$ in Equation 1. An increase in this ratio enhances the thermodynamic activity of the active ingredient in the formulation. To accomplish this, the API concentration in the vehicle needs to be augmented, i.e., increasing c_v, and/or the API solubility in the formulation needs to be diminished, i.e., reducing $c_{s,v}$. Both approaches cause an enhanced skin penetration of the active ingredient as a result of its increased thermodynamic activity [79]. Propylene glycol and oleic acid represent two examples that influence this parameter [95,96]. However, as soon as the drug concentration exceeds the saturation concentration, a suspension may be formed, and no further increase in penetration can be achieved [79,97]. This further rise can only be realized by using supersaturated

formulations. Since these formulations are thermodynamically very unstable, though, crystallization of the active ingredient is very likely to occur [89,98].

The second approach focuses on the solubility enhancement of the drug in the skin, i.e., increasing $c_{s,m}$ in Equation 1. It is supposed that propylene glycol and ethanol are examples of substances acting as penetration enhancers in this way [79]. Moreover, it has also been reported that the alkanediols studied in this work may enhance the solubility of the drug in the skin [59]. The penetration enhancers are themselves absorbed into the SC, where they increase the solubility of the active ingredient. Thus, the solubility parameter of the SC is shifted in the direction of the API by the effect of the penetration enhancer [99,100]. For some substances, a solvent drag effect also seems to be involved, wherein penetrating enhancer molecules act as a kind of carrier and drag dissolved drug molecules into the skin [95,101].

The third option to increase the penetration of an active ingredient is to modulate the barrier function of the SC. This option increases the diffusion coefficient D_m in Equation 1. The microstructure of the SC can be influenced by an interaction with the hydrophilic head groups as well as the lipophilic parts of the SC lipids and the interaction with the keratin of the corneocytes [69,73]. Penetration enhancers, such as fatty acids including oleic acid [102], azone [103] or surfactants like sodium lauryl sulfate [104] intercalate into the SC lipids. This behavior is attributed to their amphiphilic structure with the polar head group and the nonpolar long alkyl chain [79], resembling that of the SC lipids. The intercalation leads to a disorder of the originally highly ordered SC lipids which results in an increase in the SC fluidity. As a consequence, the barrier function is disturbed and the drug penetration is enhanced [105]. More hydrophilic substances, such as propylene glycol, could be deposited in the hydrophilic head groups of the lipids, since their amphiphilicity is not sufficient for intercalation into the lipophilic regions of the SC lipids. The associated rearrangement of the lipid bilayers also produces a penetration-enhancing effect [73,106]. In addition, propylene glycol is described to solvate keratin and to occupy hydrogen bonding sites for the API in the SC, and thus reducing the binding between the API and the tissue [69].

The three options presented for increasing the penetration cannot be clearly distinguished from each other. On the contrary, many penetration enhancers act in several ways as exemplified by propylene glycol.

The most common chemical penetration enhancers are summarized in Table 4 along with corresponding examples.

The safest and most commonly used method to increase the skin penetration of both lipophilic and hydrophilic active ingredients is the hydration of the SC. This can be achieved by occlusion, e.g., with components of ointments or the application of an occlusive dressing. The compact structure of the horny layer is thus fluidized, leading to enhanced drug penetration [94,107,108].

Table 4. Classes of penetration enhancers used for dermal drug delivery [93,109].

Type	Example
Water	
Alcohols	Ethanol, isopropyl alcohol
Glycols	Propylene glycol, 1,2-pentanediol, 1,2-hexanediol, 1,2-octanediol, 2-methly-2,4-pentanediol
Fatty acids	Caprylic acid, capric acid, lauric acid, linoleic acid, linolenic acid, oleic acid
Esters	Ethyl oleate, glyceryl monooleate, glyceryl monocaprylate, isopropyl myristate, sorbitan monooleate
Sulfoxides	Dimethyl sulfoxide
Surfactants	Sodium lauryl sulfate, polysorbates
Terpenes	Farnesol, limonene, menthol
Others	Azone, n-methyl-2-pyrrolidone

Since penetration enhancers partially exert their effect by being absorbed and altering the skin's barrier function, skin irritation is a common consequence, limiting the use of enhancers in some cases. However, the ideal skin penetration enhancer should be pharmacologically inert and neither toxic nor irritant or allergenic and should only have a reversible effect on the skin. Moreover, a penetration enhancer for widespread use should not be too expensive [109].

Although a number of chemical penetration enhancers are available, research is ongoing to identify candidate substances that combine as many of the desired properties as possible [110].

1.3.4. Skin Models

The objective of ex vivo studies on dermal drug absorption is to obtain results as realistic as possible which can be transferred to the in vivo behavior in the human skin. Therefore, human skin is most suitable for conducting dermal drug absorption experiments [111]. Excised human skin is usually gathered from plastic surgery or from cadavers, with abdominal, breast or back skin being the most applicable options owing to their large surface areas. However, in the evaluation it should be considered that there are significant differences in skin absorption between the various body sites [112,113]. Though, based on both the limited availability of human skin and ethical considerations, the use of animal skin or synthetic membranes having properties similar to human skin is desired for performing absorption studies [114,115].

Currently, a lot of research activity is ongoing in the field of membranes [112,115]. Artificial and synthetic membranes can be fabricated with constant properties, in particular with a uniform thickness. Further, membranes can be easily stored. In addition, membranes are inert and permeation data can be measured with a high reproducibility. However, artificial and synthetic membranes lack the heterogeneity and complexity of the human skin. The barrier function of the SC cannot be mimicked with these membranes. Consequently, correlations between permeation data obtained by using membranes and human application can hardly be established [115].

For this reason, the utilization of animal skin is still superior. However, the animal model needs to be physiologically, biochemically and anatomically similar to the human skin in order to be able to reliably transfer data extracted from animal skin experiments to the absorption behavior into human skin [116]. Skin sources of animal origin are presently mainly rodent and pig skin.

Despite rats being considered the preferred rodent model, the differences between rat skin and human skin are significant. Compared to human skin, the SC and epidermis of rat skin are considerably thinner. In addition, rat skin possesses a larger number of skin appendages. Moreover, the intercellular lipid composition of the SC differs and the corneocyte surface area is smaller than that of human skin [111].

Thus, pig skin is the preferred animal skin model [117], as it exhibits several similarities to human skin. With respect to the total porcine skin, the tissue of the porcine ear skin

has the highest similarity to human skin in terms of thickness of the different skin layers. Additionally, the structure of the SC and epidermis of pig ear skin as well as its lipid composition resemble human skin [118,119]. Furthermore, the structure of hair follicles and sweat glands are comparable to human skin [119]. Although the diameters of both hair and infundibula openings are larger in porcine ear skin than in human skin [119], porcine skin is considered the preferred skin model when human skin is not available. Penetration into and permeation through porcine skin are of a similar order of magnitude to human skin. This allows appropriate correlations to be established between the data obtained with porcine skin and the human skin [117].

1.3.5. Release, Permeation, and Penetration Models

For drug development as well as for the finished product release and shelf life specification of dermal products, it is important to develop and perform appropriate tests for in vitro release, skin penetration and permeation. The release of an API from a dermal dosage form is determined through a synthetic membrane into an acceptor medium. On the other hand, suitable skin models, as described in chapter 1.3.4., are employed for experiments of penetration, i.e., the entrance of an API into the skin, and tests of permeation, i.e., the passage of an API through the skin, respectively [120].

For all these tests, diffusion cells, such as the Franz diffusion cell, have become the gold standard [121,122]. The majority of these cells are basically similar in design and consist of an acceptor compartment with a sampling arm, a membrane and a donor compartment [123]. The benefit of Franz cells is to allow release studies as well as penetration and permeation experiments to be performed by selecting a suitable membrane or a skin sample without modifying the principal setup.

The enhancer cell [124] and the flow-through cell [125] are additionally available for release studies of dermal products. However, they are not suited for permeation and penetration experiments. To this end, a skin PAMPA (Parallel Artificial Membrane Permeability Assay) has been developed as a fast and cost effective model which has been widely used in the past years to determine the skin permeation of a drug in order to estimate its penetration [126,127]. Since the skin PAMPA does not reflect the biological complexity of the skin, its application is so far limited to the early development phase and pre-screening of new drug candidates and formulations [115].

For the evaluation of release and permeation studies, samples are taken from the acceptor medium at specific time points and analyzed directly for the drug content, commonly by using HPLC (High Performance Liquid Chromatography) [128]. For the evaluation of penetration studies, however, the skin needs to be segmented after incubation in order to analyze the penetration profile. Frequently, methods such as tape stripping or cryosection are employed for this purpose [129,130]. However, the assessment of penetration profiles using these methods requires a skin sample for each time point, since the respective skin must be destroyed to analyze the drug content. For this reason, many new approaches target non-destructive real-time measurements of the penetration, such as two-photon scanning fluorescence microscopy [131] or confocal Raman spectroscopy (CRS) [132,133]. By means of CRS, information is obtained about the molecular structure of both the skin components and the API applied. Therefore, in addition to penetration studies, this metrology tool allows the analysis of modifications of skin structures, which makes it a promising technique for investigating and understanding the skin penetration of APIs [121,134,135].

1.4. Triamcinolone Acetonide

Triamcinolone acetonide (TAA) is a synthetic glucocorticoid whose structure is shown in Figure 6. It is administered dermally for the treatment of inflammatory, allergic and pruritic dermatoses and joint inflammation in rheumatic and degenerative diseases [136] as well as periocularly and intravitreally for the treatment of retinal vasculature disease and uveitis [137]. Furthermore, TAA is also used systemically for bronchial asthma, allergies and skin diseases, e.g., contact dermatitis and psoriasis [136].

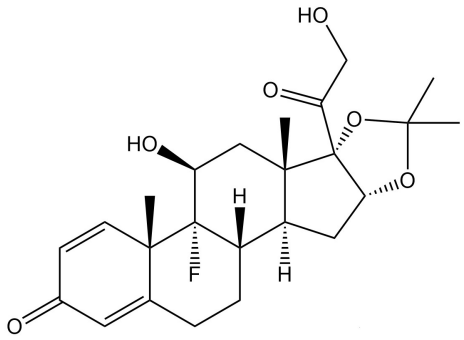

Figure 6. Chemical structure of TAA.

TAA penetrates the cell membrane and binds to the corticosteroid receptor in the cytoplasm due to its lipophilicity with a log($P_{octanol\text{-}water}$) of 2.53 [138]. The binding of TAA to the corticosteroid receptor inhibits or stimulates the regulation of the transcription of various genes, consequently causing a decreased expression of adhesion molecules, a restriction of T-cell migration, a reduction of proinflammatory cytokine synthesis, and a stimulation of the lipocortin synthesis. These modifications result in anti-inflammatory, antiproliferative, immunosuppressive, and vasoconstrictive effects [139,140].

In contrast to hydrocortisone, which was the first corticosteroid widely used in dermatology [141] and is classified as least potent, TAA is considered medium to high potent, depending on the dosage vehicle [17,142]. Its increased activity over hydrocortisone is attributed to the additional double bond between C1 and C2 [141]. Unlike triamcinolone, which lacks the 16α, 17α ketal with acetone, triamcinolone acetonide is much more lipophilic. Therefore, despite approximately the same systemic potency, TAA is ten times more potent when applied topically to the skin compared to triamcinolone. These characteristics predestine the usage of TAA in dermal dosage forms [136].

The glucocorticoid receptors which have to be addressed are located in the epidermal and dermal cells [3]. Hence, once the formulation is applied to the skin, TAA first has to overcome the SC to reach its target structures. However, it penetrates only slowly through the SC as a consequence of its pronounced lipophilicity [136]. This causes an accumulation in this layer and the formation of a reservoir. TAA is released therefrom over a prolonged period of time [143]. Thus, dermal preparations containing TAA are usually applied only once per day [144]. Several applications per day are advisable in the case of severe dermatosis, in which the SC is damaged to such an extent that an adequate TAA depot cannot be built up [145].

As previously stated, due to the localization of its targets, it is necessary for TAA to permeate the SC and achieve sufficient concentrations in the living layers of the epidermis to maximize the therapeutic effect. To this end, penetration enhancers can be employed. However, systemic concentrations should be as low as possible to minimize side effects [146]. Local reactions such as cutaneous atrophy, acne-like symptoms, and facial inflammatory skin lesions may occur associated with dermal TAA application. However, owing to its high lipophilicity, systemic side effects are less frequent [147].

The topical administration of TAA is widespread. It is used in both dermal proprietary medicinal products, comprising solutions, emulsions, creams and ointments [1,148], as well as in extemporaneous preparations, in a standard application concentration of 0.1% [136]. A hydrophilic TAA cream based on BC is monographed in the NRF in concentrations of 0.025%, 0.05% and 0.1% [145]. In addition, the NRF describes a hydrophilic TAA emulsion for dermal application with the same concentrations as those in the cream [149]. The NRF formulation guidelines also include the combination of TAA with urea, salicylic acid, chlorhexidine digluconate and ammonium bituminosulfonate [9].

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2. Aim

Alkanediols are frequently used as multifunctional ingredients in dermal preparations. Primarily, they are utilized as alternative preservatives owing to their antimicrobial properties. These antimicrobial features are attributed to their amphiphilic structure, which allows the alkanediols to be incorporated into membranes of microorganisms.

However, their chemical structure also seems to predispose these substances to interact with the components of the formulation applied and to modify the percutaneous penetration of drugs. Further, as outlined above, alkanediols have moisturizing and solubilizing properties, which may have an effect on the drug absorption into the skin. Accordingly, the usage of these multifunctional substances may cause various interactions with the formulation on the one hand and the skin on the other hand. These interactions induced by the alkanediols have to be analyzed in detail in order to apply these substances in a controlled manner. Therefore, a number of open items must be clarified prior to enable the widespread use of alkanediols as ingredients, in particular as alternative preservatives, in semisolid dermal preparations:

(I) It is necessary to obtain a comprehensive understanding of the magnitude to which alkanediols alter the properties of the formulations. The application of alkanediols in a dermal preparation should not be promoted, if the dermal formulation is modified to an extent which cannot be tolerated or if the shelf-life of the dermal formulation is significantly reduced.

(II) Further, the preservative effect of alkanediols in different formulations must be assessed quantitatively in order to ensure their appropriate microbiological quality.

(III) Moreover, for the use in medicinal products, it is essential to precisely know the extent to which alkanediols alter the dermato-biopharmaceutical properties of the preparation. Since drug release from semisolid dermal dosage forms is considered to be an important quality attribute, the changes caused by alkanediols must be examined.

(IV) In addition, for a safe application, the modification of the skin penetration and permeation of an active ingredient induced by the alkanediols must be analyzed and assessed.

To clarify these issues, as a preliminary study, the interfacial activity of four different alkanediols was evaluated, namely 2-methyl-2,4-pentanediol, 1,2-pentanediol, 1,2-hexanediol and 1,2-octanediol. The results of the interfacial activity study are displayed in chapter 3 of this thesis.

Subsequently, for addressing the first item, the alkanediols were incorporated into a classic hydrophilic cream for examining their insertion into the inner cream structures as well as for detecting a possible instability of the cream caused by this incorporation. Chapter 4 presents the findings of these investigations.

Further, for elucidating the second point, the preservative effect of the alkanediols was assessed by performing a test of efficacy of antimicrobial preservation according to the Ph. Eur. The outcome of this study is also described in chapter 4.

The major topic of this work concentrates on the biopharmaceutical characterization of drug-containing preparations in order to resolve the third and fourth item indicated above. The glucocorticoid TAA was chosen as a model drug substance. This choice was motivated by its broad application both in extemporaneous formulations as well as in finished drug products. Besides, TAA was also selected due to fact that its target structures are localized in the living cell layers, requiring the SC to be overcome. Therefore, TAA is excellently suited for studying the influence of alkanediols on its drug release as well as on its skin penetration and permeation. Additionally, four different semisolid vehicles were selected for the study that are commonly used in custom compounded formulations, in order to examine the effect of the base on the biopharmaceutical characteristics.

To answer the question of the third item, chapter 5 focuses on the alteration of the TAA release from different formulations resulting from the addition of alkanediols.

To respond to the fourth issue, chapter 6 addresses the modification of the TAA skin penetration and permeation. The impact of alkanediols on the content and the structural arrangement of the SC lipids was analyzed, to consider the penetration-enhancing effect of the alkanediols in detail.

In summary, it is the objective of this thesis to investigate the effects of alkanediols on various properties of semisolid dermal dosage forms in order to deduce their suitability

as alternatives for conventional preservatives, as well as to elaborate relevant factors to be considered in their application.

3. Interfacial Activity of the Alkanediols

3.1. Abstract

Due to their versatile properties, alkanediols are often used as alternative preservatives in dermal formulations. Their antimicrobial effect is attributed to their chemical structure. Likewise, possible interactions with components of the semisolid formulation applied are related to the structure of the alkanediols. Additionally, it is known that alkanediols can interact with the lipids of the stratum corneum (SC), resulting in a penetration-enhancing effect. It is assumed that these properties are related to the amphiphilicity of the alkanediols. Therefore, the interfacial activity of four different alkanediols was investigated in this study. For this purpose, the interfacial tensions of aqueous alkanediol solutions with liquid paraffin were measured using pendant drop shape analysis.

The results reveal a decreasing interfacial tension with increasing alkanediol concentration. Further, the shorter-chained alkanediols, 2-methyl-2,4-pentanediol and 1,2-pentanediol, showed a low interfacial activity, while the longer-chained alkanediols, 1,2-hexanediol and 1,2-octanediol, were more surface-active.

The experimental data demonstrates that the amphiphilicity of alkanediols augments with increasing chain length. Therefore, it is likely that the antimicrobial effect, the interaction with the surfactants of the formulation used as well as with the SC lipids depend on the chain length of the alkanediols.

3.2. Introduction

Alkanediols are frequently used as multifunctional excipients in various dermal formulations. In addition to their moisturizing, solubilizing and penetration-enhancing properties, these substances also exhibit antimicrobial activity. Therefore, alkanediols are often employed as alternative preservatives [1–4].

The most widely used compounds are 2-methyl-2,4-pentanediol, 1,2-pentanediol, 1,2-hexanediol, and 1,2-octanediol whose chemical structure is composed of two hydroxy groups and a lipophilic alkyl residue of different length.

Their amphiphilic structure enables alkanediols to penetrate the lipid bilayer of microbial membranes. Consequently, the transport through the membranes is inhibited

and essential cellular components leak out. A longer alkyl chain of the alkanediol favors membrane penetration, and thus results in a greater disruptive effect [5].

Preservatives are needed in the aqueous phase of the formulation, because microorganisms are able to proliferate therein. Therefore, preservatives must be sufficiently soluble in water to reach a concentration in the aqueous phase high enough to inhibit the development of microorganisms. However, if the alkyl residue of alkanediols exceeds a certain length, their water solubility diminishes to such an extent that the preservative effect decreases. This reduction in the preservative effect occurs although the antimicrobial effect of alkanediols with a high number of carbon atoms is strong [5]. For this reason, alkanediols having an alkyl chain longer than that of 1,2-octanediol are not applied for preservation.

In addition to their antimicrobial activity, the amphiphilic nature of alkanediols suggests a high probability of interaction with the components of dermal formulations [2]. Hence, viscosity, stability and appearance of dermal formulations may be altered when adding alkanediols for preservation.

Moreover, it has been shown that alkanediols interact with the lipids of the SC owing to their specific structure [6]. This may cause a penetration enhancement [4,7], which has to be considered when using alkanediols in dermal medicinal products.

As the amphiphilicity of these substances may have profound consequences on their use as alternative preservatives in dermal dosage forms, the interfacial activity of the respective alkanediols was investigated in the present study.

3.3. Material and Methods

3.3.1. Materials

1,2-Pentanediol, 2-methyl-2,4-pentanediol, and 1,2-hexanediol (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany), liquid paraffin (Hansen & Rosenthal KG, Hamburg, Germany). 1,2-Octanediol (dermosoft Octiol) was kindly donated by Evonik Dr. Straetmans GmbH, Hamburg, Germany. Ultrapure water (Elga Maxima, High Wycombe, United Kingdom) was used to prepare the solutions.

3.3.2. Preparation of Aqueous Alkanediol Solutions

The aqueous alkanediol solutions to be measured were freshly prepared on the day of the measurement. 0.05%, 0.1%, 0.2%, 0.5%, 1.0%, 3.0% and 5.0% solutions of each alkanediol were produced by mixing the corresponding alkanediol with ultrapure water using a magnetic stirrer. Solutions of 1,2-octanediol could only be measured in concentrations up to its saturation solubility which is 1.82% [8].

3.3.3. Determination of the Densities of the Solutions

The required densities of the hydrophilic and lipophilic phases for the following interfacial tension measurements were determined by means of a DMA 4500 Density Meter (Anton Paar, Graz, Austria) at a temperature of 23 °C. The determination was carried out in triplicate.

3.3.4. Measurement of the Interfacial Tensions

The measurements of the interfacial tensions between aqueous alkanediol solutions having different concentrations and liquid paraffin were performed using a Profile Analysis Tensiometer (PAT) device (Sinterface Technologies, Berlin, Germany). The fundamental principle is the drop shape analysis of a pendant drop generated in a phase that is immiscible with the drop liquid. The interfacial tension is determined by analyzing the measured drop shape. This is achieved by performing a fitting procedure (PAT 1 Fitting Version 5.06, Sinterface Technologies, Berlin, Germany) subsequent to the measurement of the drop shape.

The calculation of the interfacial tension is based on the Young-Laplace equation (Equation 1) [9].

$$\gamma\left(\frac{1}{R_1}+\frac{1}{R_2}\right) = \Delta P_0 + \Delta\rho g h \qquad (1)$$

In Equation 1 γ represents the interfacial tension. It is defined by the energy necessary to increase the surface area of the drop [$J \cdot m^{-2} = N \cdot m^{-1}$]. R_1 and R_2 describe the principal radii of curvature [m] of the drop, $\triangle P_0$ denotes the pressure difference in a reference plane [$N \cdot m^{-2}$], Δρ is the density difference between the density of the liquid to be investigated and the density of the reference medium [$kg \cdot m^{-3}$], g stands for the acceleration of gravity [$m \cdot s^{-2}$] and h describes the vertical drop height [m].

In the present study, the shape of the pendant drop was recorded by an integrated CCD (charge-coupled device) camera at one frame per second for a period of 900 s. Due to the lower density of the liquid paraffin in comparison to the aqueous solutions, the liquid paraffin was placed in a quartz cuvette and served as external phase. It was tempered to 23 °C prior to the start of the experiment. The hydrophilic pendant drop consisted of alkanediol solutions of different concentrations, wherein five drops of each solution were generated and measured, respectively. The drop volume was kept constant at a very high level, close to the critical detachment volume, in order to obtain precise measured values [10]. The interfacial tension between liquid paraffin and pure water was measured as a reference.

As a result of the fitting process, the dynamic interfacial tension was obtained. Since the adsorption to the interface is a time-dependent process, the interfacial tension decreases over time. In the present experiments γ was found to remain almost constant over the last 300 s of the measurement period of 900 s. The equilibrium interfacial tension corresponds to an extrapolation of the dynamic interfacial tension to an infinite measurement time. Therefore, its value is obtained as the y-axis intercept when plotting the interfacial tension against $1/\sqrt{t}$ and calculating the regression line from 600 to 900 s. This evaluation is demonstrated in Figure 1.

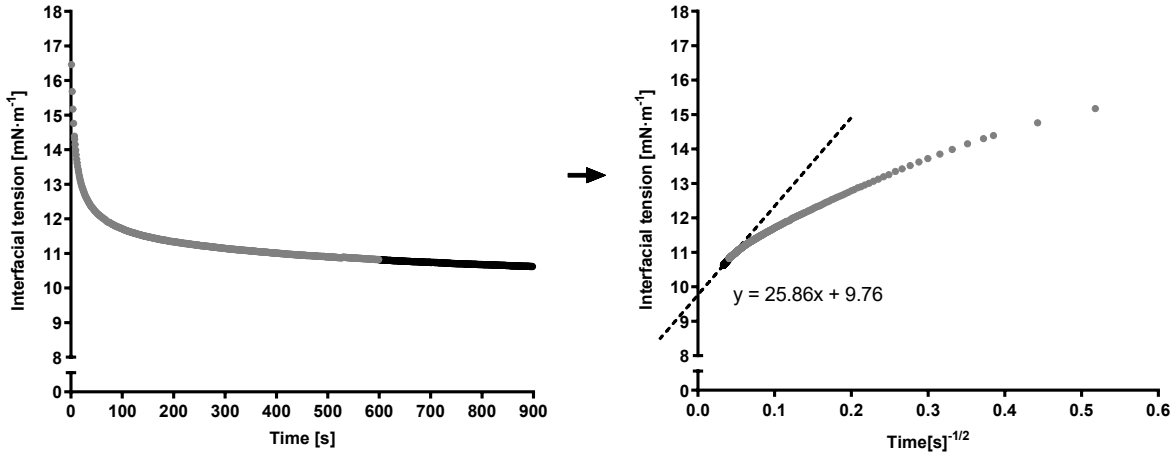

Figure 1. Evaluation of the dynamic interfacial tension to the equilibrium interfacial tension by a linear regression of the values of the last 300 s extrapolated to the y-axis. In this example: equilibrium interfacial tension = 9.76 $N \cdot m^{-1}$.

3.3.5. Statistical Analysis

The following graphs represent the arithmetic mean ± standard deviation extracted from fivefold measurements. Statistical differences in the equilibrium interfacial tension of water and the 5% concentrated solutions of 2-methyl-2,4-pentanediol, 1,2-pentanediol and 1,2-hexanediol were analyzed with an one-way ANOVA (analysis of variance) followed by Tukey's multiple comparisons test. The significant difference is shown with asterisks (*), with **** denoting $p \leq 0.0001$.

3.4. Results and Discussion

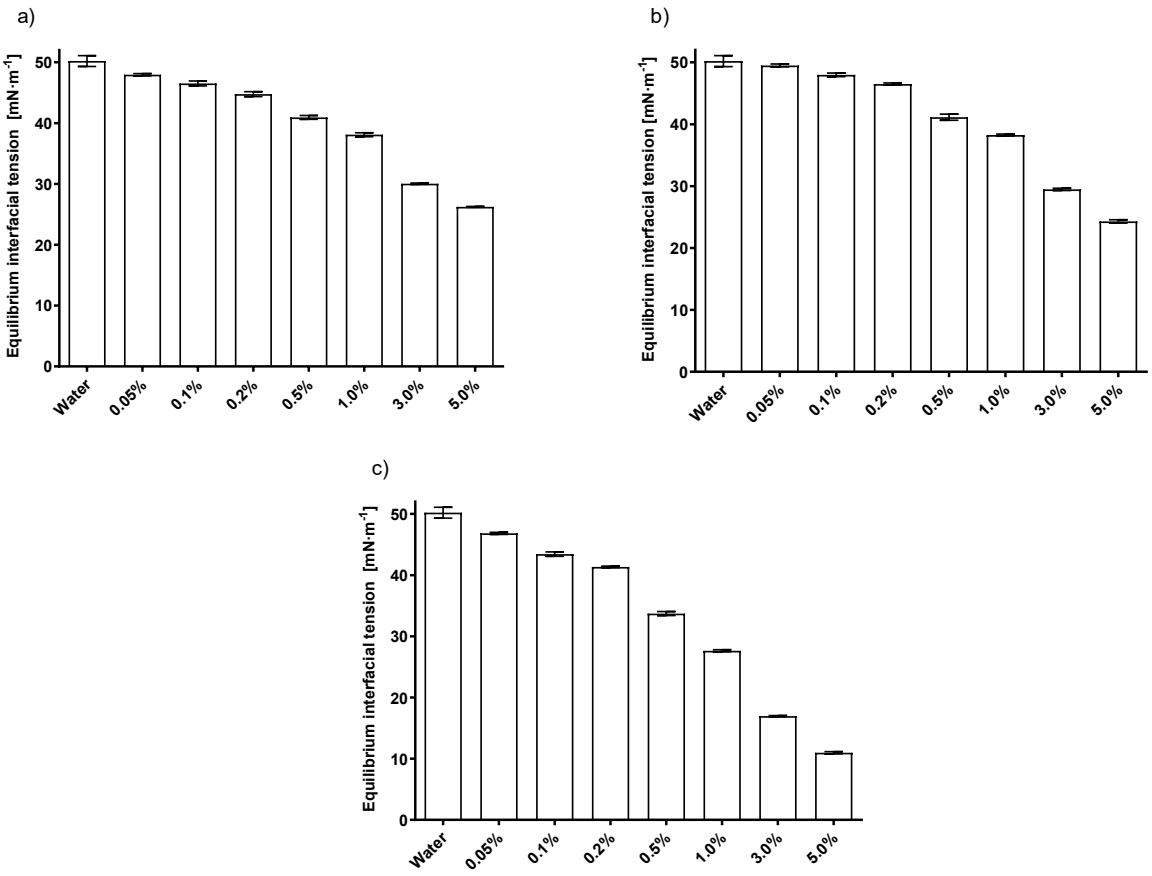

Figure 2. Equilibrium interfacial tensions between solutions having different concentrations of a) 2-methyl-2,4-pentanediol, b) 1,2-pentanediol, c) 1,2-hexanediol and liquid paraffin, n = 5, mean ± standard deviation.

Figure 2 depicts the equilibrium interfacial tensions between various aqueous alkanediol solutions and liquid paraffin. In all graphs, the interfacial tension of pure water is presented as a reference value. For each alkanediol it can be observed that the interfacial tension decreased with increasing alkanediol concentration. The variation of the interfacial tension for solutions containing 2-methyl-2,4-pentandiol and

1,2-pentanediol is similar, whereas the interfacial tension of solutions containing 1,2-hexanediol showed a significantly stronger dependence on concentration. Thus, 1,2-hexanediol considerably reduced the interfacial tension in particular in higher concentrations.

For 1,2-octanediol solutions, no results for the equilibrium interfacial tension can be plotted. This is a consequence of the steady increase of the dynamic interfacial tension with time in the experiments after having passed through a minimum in the first seconds. A typically measured curve is shown in Figure 3. This phenomenon can be explained by the longer alkyl chain, increasing the solubility of 1,2-octanediol in the lipophilic phase. Consequently, 1,2-octanediol will migrate to a certain extent into the liquid paraffin phase [11,12]. As a distribution equilibrium is not reached within the measuring cycle, no equilibrium interfacial tension can be determined. Irrespectively, very low equilibrium interfacial tensions (< 7.3 $mN \cdot m^{-1}$) could be calculated by means of the drop shape analysis when the 1,2-octanediol concentration was < 1%.

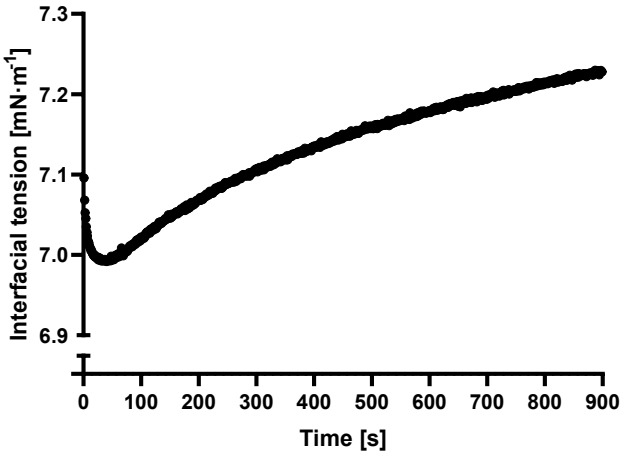

Figure 3. Typical course of dynamic interfacial tension between 1,2-octanediol solutions in concentrations of < 1% and liquid paraffin (in the presented example the concentration was 0.5%).

At concentration ≥ 1%, the interfacial tension of the aqueous 1,2-octanediol solutions with liquid paraffin were too low to reveal stable drops. Rather, the drops broke off immediately after their formation. Therefore, measurements of the dynamic interfacial tension could not be executed for concentrations > 1% 1,2-octanediol.

Figure 4 compares the respective interfacial tensions between 5% aqueous solutions of 2-methyl-2,4-pentanediol, 1,2-pentanediol and 1,2-hexanediol and liquid paraffin. Again, the interfacial tension of pure water is displayed as a reference value.

1,2-Octanediol is not contained in Figure 4 for the reason discussed above. It is evident from this figure that the various alkanediols differ significantly in their impact on the interfacial tension. The 2-methyl-2,4-pentanediol solution exhibited the smallest effect reducing the interfacial tension by 24.0 $mN \cdot m^{-1}$. The influence of 1,2-pentanediol on the interfacial tension was slightly stronger than that of 2-methyl-2,4-pentanediol. 1,2-Hexanediol had the most pronounced effect and reduced the interfacial tension of paraffin with water by 39.2 $mN \cdot m^{-1}$ which was nearly twice compared to the two other alkanediols.

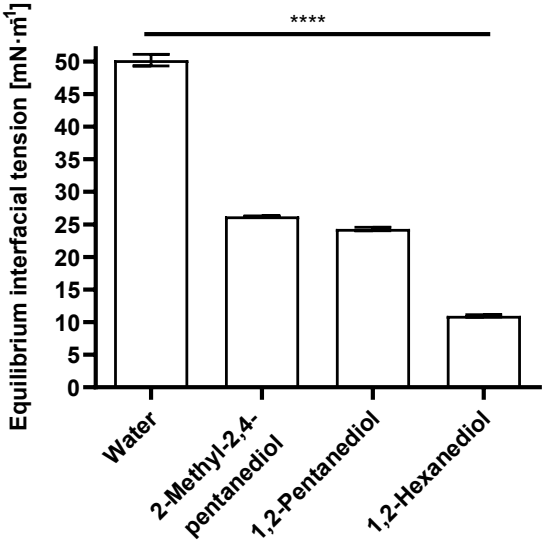

Figure 4. Comparison of interfacial tensions between water and 5% solutions of 2-methyl-2,4-pentanediol, 1,2-pentanediol and 1,2-hexanediol and liquid paraffin, n = 5, mean ± standard deviation.

The obtained results can be directly related to the structure of the alkanediols investigated. While 1,2-pentanediol and 2-methyl-2,4-pentanediol have an alkyl chain consisting of five carbon atoms, the chain length of 1,2-hexanediol is extended by one carbon atom. As a result, the amphiphilicity of 1,2-hexanediol is enhanced, causing a higher interfacial activity.

The position of the hydroxy groups in 2-methyl-2,4-pentanediol deviates from that of the other alkanediols studied, since they are not at positions 1 and 2, but at 2 and 4. This structural difference is reflected by the interfacial tension. 2-Methyl-2,4-pentanediol exhibited the highest value of interfacial tension and thus showed the lowest interfacial activity. This means that, the positioning of the hydroxyl groups along the molecule, rather than at one end, leads to a reduction in amphiphilicity.

Although no exact numbers can be reported for 1,2-octanediol solutions, it can be deduced that this substance is highly surface-active. The fact that no drop can be formed in concentrations ≥ 1% contributes to the conclusion that the interfacial activity of 1,2-octanediol is the highest of all measured alkanediols. This is consistent with the longer alkyl chain of this alkanediol, which comprises eight carbon atoms. Consequently, 1,2-octanediol has the highest amphiphilicity of all investigated alkanediols.

3.5. Conclusion and Outlook

In the present study, the interfacial tensions between various aqueous alkanediol solutions and liquid paraffin were determined. For all tested alkanediols, the interfacial tension was found to decline with increasing alkanediol concentrations in the aqueous solution.

Further, the interfacial tension varies between the different alkanediols investigated. 2-Methyl-2,4-pentanediol and 1,2-pentanediol showed the lowest interfacial activity among the alkanediols studied. 1,2-Hexanediol was found to reduce the interfacial tension of paraffin with water about twice as much as the shorter-chained alkanediols.

The equilibrium interfacial tensions of the aqueous 1,2-octanediol solutions, however, were too low to be measured using pendant drop shape analysis. This can be attributed to a substantial solubility of 1,2-octanediol in liquid paraffin. As the adsorption to the interface was thus superimposed to the partition of the substance into the lipid phase, no equilibrium interfacial tension could be measured. Nevertheless, a high interfacial activity of this alkanediol can be supposed, since some values below 7.3 $N \cdot m^{-1}$ were assessed, and no stable drops could be generated when the 1,2-octanediol concentration in the aqueous solution was ≥ 1%.

These results are a direct consequence of the chemical structures of the alkanediols. Since the amphiphilicity and thus the interfacial activity augments with increasing chain length of the alkyl residue of the substances, the interfacial tension reduces as a function of the alkyl chain length.

As the antimicrobial effect also correlates with the amphiphilicity of the alkanediols studied, 1,2-octanediol is the one having the highest antimicrobial activity [1,5]. This is also consistent with our studies which determine an increased interfacial activity. This

enlarged interfacial activity allows for improved incorporation into the membranes of microorganisms.

Moreover, it can be deduced that the use of alkanediols as alternative preservatives for dermal products may cause an interaction with surfactants and co-surfactants used to stabilize creams or emulsions. The presented results indicate that this interaction may intensify with increasing chain length of the alkanediols as a consequence of their amphiphilicity.

The amphiphilic nature of alkanediols detected in this work may lead to interactions of alkanediols with the SC lipids when used in dermal formulations, as revealed in a previous study [6]. This interaction potentially causes a penetration-enhancing effect of the tested alkanediols.

Overall, the results suggest that using alkanediols as alternative preservatives may affect both the stability of the formulations as well as the drug penetration as a result of their amphiphilic nature. Therefore, these aspects were analyzed in detail in the course of the present work and are presented in the following chapters.

3.6. References

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4. Investigations on Alkanediols as Alternative Preservatives in a Nonionic Hydrophilic Cream

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4.1. Abstract

Alkanediols are often used as alternative antimicrobial preservatives for dermal formulations, e.g., hydrophilic creams. These substances show an antimicrobial effect due to their amphiphilic structure. At the same time, their amphiphilic behavior enables various interactions with the cream base itself. Therefore, the effect of four different alkanediols, namely 1,2-pentanediol, 2-methyl-2,4-pentanediol (hexylene glycol), 1,2-hexanediol, and 1,2-octanediol on the physical stability of a nonionic hydrophilic cream was investigated. Further, the incorporation of the alkanediols into lamellar structures of the cream was evaluated using differential scanning calorimetry (DSC) and small-angle X-ray scattering (SAXS) measurements. The interaction with the mixed crystals of the cream was found to increase with raising alkyl chain length of the added alkanediol. As a result, consistency and stability of the cream are slightly impaired. A test for efficacy of antimicrobial preservation according to the European Pharmacopoeia (Ph.Eur.) revealed that the antimicrobial activity is directly linked to the length of the alkyl chain of the alkanediols. 2-Methyl-2,4-pentanediol differs from both findings. This compound has non-vicinal hydroxy groups which result in a reduced amphiphilicity compared to the others. Consequently, it has a smaller impact on the colloidal structure of the cream and shows a comparatively low antimicrobial activity.

Keywords: alkanediols; preservation; amphiphilicity; rheology; DSC; X-ray

4.2. Introduction

Many commonly used antimicrobial preservatives in dermal formulations are increasingly under discussion, especially when they are used in pediatric formulations [1]. Moreover, some preservatives, e.g., parabens, are under debate due to their endocrine disrupting properties [2]. Hence, there is a growing need for alternative compounds for antimicrobial preservation in order to meet the microbiological quality acceptance criteria for cutaneous preparations. Alkanediols could be candidates for substituting conventional preservatives. The chemical structures of the alkanediols used in this study are presented in Figure 1.

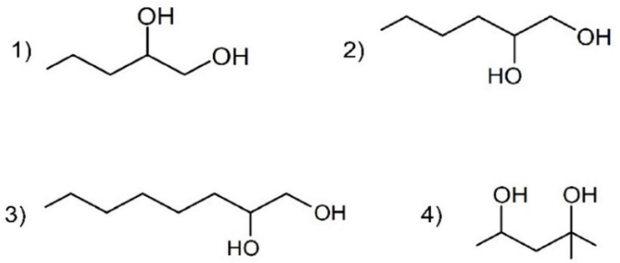

Figure 1. Chemical structures of the alkanediols used: (1) 1,2-pentanediol, (2) 1,2-hexanediol, (3) 1,2-octanediol, (4) 2-methyl-2,4-pentanediol.

1,2-Pentanediol, 1,2-hexanediol, and 1,2-octanediol are 1,2-alkanediols, having vicinal hydroxy groups on C1 and C2 positions. 2-Methyl-2,4-pentanediol, however, differs from this basic structure as it has two hydroxy groups on positions C2 and C4. The preservative action of these substances is ascribed to their amphiphilic properties which enable their integration into the cell membranes of micro-organisms [3]. Without having acidic or basic functional groups, alkanediols are antimicrobially effective over a wide pH range [4]. In addition to their antimicrobial effectiveness, these substances also exhibit moisturizing properties and are thus used as multifunctional excipients in cosmetic formulations [5].

As a consequence of their amphiphilic structure, alkanediols also interact with the formulation itself [6]. To investigate this interaction, alkanediols were incorporated into a commonly used base in custom compounded creams. As an example, the nonionic hydrophilic cream (NHC) according to the German Pharmacopeia (DAB) was chosen. Its colloidal structure has been described in detail. Figure 2 schematically depicts its structure as proposed by Junginger [7]. The mixed crystal (A) is formed of polysorbate 60 and cetostearyl alcohol. Water can be accommodated and immobilized (B) within

the lamellae of this mixed crystal. Both, the mixed crystal (A) and the immobilized water (B), together form the hydrophilic gel phase. The remainder of the cetostearyl alcohol which crystallizes as hemihydrate forms the lipophilic gel phase (C) which encloses the dispersed lipophilic phase (E). The bulk water phase (D) is immobilized within the coherent matrix structure of the two gel phases.

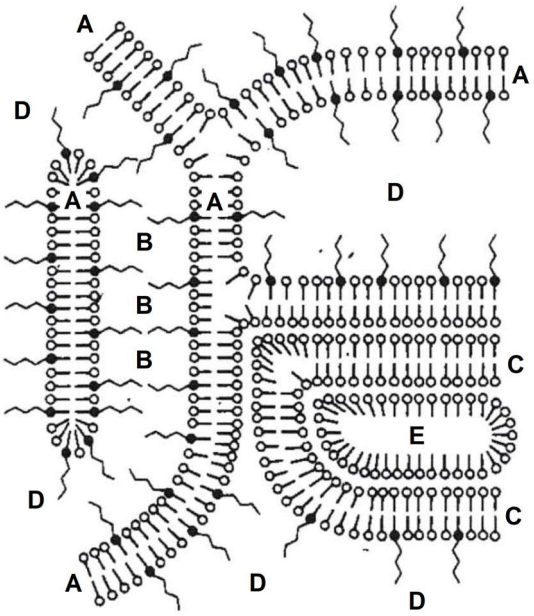

Figure 2. Model structure of the nonionic hydrophilic cream (NHC) according to the German Pharmacopeia (DAB) modified according to Junginger [7]: (A) mixed crystal of polysorbate 60 and cetolstearyl alcohol, (B) interlamellarly fixed water, (C) lipophilic gel phase, (D) bulk water, (E) lipophilic dispersed phase.

It was the objective of the present investigations to analyze the effect of several alkanediols on the physical stability of NHC. Further, the incorporation of the alkanediols into the lamellar structures of the cream was evaluated using DSC and SAXS measurements. Finally, the efficacy of antimicrobial activity was tested.

4.3. Materials and Methods

4.3.1. Materials

1,2-Pentanediol, 2-methyl-2,4-pentanediol, and 1,2-hexanediol (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany), polysorbate 60 (Kolliphor PS 60, BASF SE, Ludwigshafen, Germany), cetostearyl alcohol (Kolliwax CSA, BASF SE, Ludwigshafen, Germany), glycerol 85% (Dr. Willmar Schwabe GmbH & Co. KG, Karlsruhe, Germany), white soft paraffin (Sasol Performance Chemicals, Hamburg, Germany), potassium sorbate (Caesar & Loretz GmbH, Hilden, Germany), citric acid

(Jungbunzlauer Suisse AG, Basel, Switzerland). 1,2-Octanediol (dermosoft Octiol) was kindly donated by Evonik Dr. Straetmans GmbH, Hamburg, Germany.

4.3.2. Preparation of Test Formulations

The nonionic hydrophilic cream DAB was prepared according to the German Pharmacopoeia [8]. It has the following composition: polysorbate 60 5% (w/w), cetostearyl alcohol 10% (w/w), glycerol 85% 10% (w/w), white soft paraffin 25% (w/w), purified water 50% (w/w).

For antimicrobial preservation, either (i) no alkanediol, (ii) 5% 2-methyl-2,4-pentanediol, (iii) 5% 1,2-pentanediol, (iv) 3% 1,2-hexanediol, or (v) 1% 1,2-octanediol related to the whole formulation was added to the hydrophilic phase at 70 °C. These amounts correspond to the highest application concentrations found in literature [9,10,11]. The quantity of alkanediol was deducted from the amount of water. Further, three formulations were prepared using sorbic acid. Formulation (is) is the commonly used cream base which is preserved with solely 0.1% sorbic acid. Formulations (iis) and (iiis) are creams which were preserved with either 2-methyl-2,4-pentanediol or 1,2-pentanediol complemented with 0.05% sorbic acid. All manufactured formulations are summarized in Table 1.

Table 1. Overview of test formulations.

Formulation	Additive	Concentration (%)
(i)	-	-
(is)	Sorbic acid	0.1
(ii)	2-methyl-2,4-pentanediol	5
(iis)	2-methyl-2,4-pentanediol + sorbic acid	5 + 0.05
(iii)	1,2-pentanediol	5
(iiis)	1,2-pentanediol + sorbic acid	5 + 0.05
(iv)	1,2-hexanediol	3
(v)	1,2-octanediol	1

After manual premixing, the formulations were finally homogenized using an Unguator 2100 mixing system (Gako International GmbH, Scheßlitz, Germany) with increasing rotation speed from 375 to 1900 rpm within 224 s.

4.3.3. Stability study

After preparation, the creams were filled in airtight glass vials and stored for 6 months at room temperature. The stored samples were characterized by oscillation rheometry,

polarization microscopy, and a centrifugation test after 1, 7, 14, 28 days, 3, and 6 months.

4.3.4. Rheological Measurements

Oscillation measurements were performed by means of a Physica MCR501 rheometer (Anton Paar, Graz, Austria) equipped with a plate-plate geometry (diameter: 25 mm; gap size: 0.2 mm). The temperature was set to 23 °C. The measuring cycle is displayed in Table 2.

Table 2. Measurement parameters of the rheological investigations.

Phase	Condition	Duration (min)
Pre-shear	Shear rate = 5 s^{-1}	1
Rest	-	2
Amplitude sweep	Logarithmically from 0.01% to 100%; 1 Hz	

The storage modulus G' in the linear viscoelastic region was determined and interpreted as a measure of the gel strength. Furthermore, the flow point was determined from an amplitude sweep as the shear stress at the cross over point (G' = G''). All measurements were performed in triplicate.

4.3.5. Polarization Microscope

Undiluted formulations were applied thinly to a glass microscope slide and covered with a coverslip. An Axio Imager Z1 microscope (Carl Zeiss, Jena, Germany) with crossed polarizers and a ¼ λ-plate was used to capture the polarization microscopic images of the samples at a magnification of 40×.

4.3.6. Centrifugation Test

A centrifugation test was performed to determine the water holding capacity of the preparations. For this purpose, 1.25 g of each formulation (i), (ii), (iii), (iv), and (v) was weighed into a centrifugation tube and centrifuged in an Eppendorf MiniSpin Centrifuge (Eppendorf AG, Hamburg, Germany) according to the scheme indicated (Figure 3). After each centrifugation step, the formulations were visually examined with respect to water separation. If no phase separation occurred, the test was continued at the next stage.

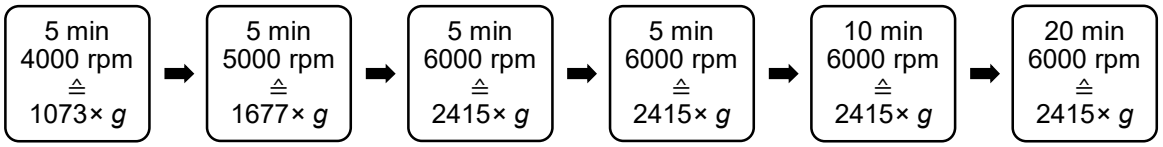

Figure 3. Diagram of the centrifugation test to determine the water holding capacity.

4.3.7. Efficacy of Antimicrobial Preservation

The efficacy of antimicrobial preservation for formulations (is), (ii), (iis), (iii), (iiis), (iv), and (v) was tested according to the European Pharmacopoeia (Ph.Eur.) 5.1.3. [12]. In brief, after ensuring sterility at the beginning, the samples were inoculated each with a suspension of one of the test organisms (*Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 6538, *Candida albicans* ATCC 10231, and *Aspergillus brasiliensis* ATCC 16404 as described in the Ph. Eur. supplemented by *Eschericha coli* ATCC 8739). The microbial count of the suspension of inoculum was adjusted to yield 10^5–10^6 colony forming units (CFU) per gram after inoculation. The inoculated products were stored at 20–25 °C, protected from light. The total microbial count was determined 2 and 28 days after inoculation using the plate count method according to Ph. Eur. 2.6.12 [12]. Additionally, the count of the viable microorganisms was carried out after 7 and 14 days if the previous measurements resulted in total microbial count values of ≥10. Otherwise, it was assumed that the values are <10. To evaluate the antimicrobial activity, the $\log_{10}$ reduction in the number of viable micro-organisms against the value obtained for the inoculum was calculated and compared with the criteria for evaluation of antimicrobial activity according to Ph. Eur., as described in Table 3.

Table 3. Criteria for evaluation of antimicrobial activity for preparations for cutaneous application according to Ph. Eur.

Microorganisms	$\log_{10}$ Reduction				
	Criteria	2 Days	7 Days	14 Days	28 Days
Bacteria	A	2	3	-	NI
	B	-	-	3	NI
Fungi	A	-	-	2	NI
	B	-	-	1	NI

NI: no increase in number of viable micro-organisms compared to the previous reading.

4.3.8. Preparation of Mixed Crystals for DSC and SAXS Measurements

The mixed crystal consists of polysorbate 60, cetostearyl alcohol, water and, if applicable, an alkanediol. For the preparation, polysorbate 60 and cetostearyl alcohol were melted at 70 °C. After heating water to the same temperature, the alkanediol was added and manually incorporated into the mixture of polysorbate 60 and cetostearyl alcohol. The mixed crystal was cooled to room temperature while stirring. The ratio of the incorporated alkanediol to the mixed crystal was the same as in the NHC prepared for the stability study.

4.3.9. Differential Scanning Calorimetry (DSC)

The melting points of the individual substances and the mixed crystals were determined by thermoanalytical measurement using a DSC 820 (Mettler Toledo GmbH, Gießen, Germany). About 20 mg of the sample were accurately weighed into a 40 µL aluminum crucible and cold sealed. The samples were cooled to −50 °C (for 1,2-hexanediol and 1,2-octanediol) or to −100 °C (for 1,2-pentanediol and 2-methyl-2,4-pentanediol), kept at this temperature for 2 min, and then heated to 80 °C, where they were again kept isothermally for 2 min. This temperature cycle was repeated a second time. The heating and cooling rate was 5 K/min, respectively. An empty crucible was used as reference. The second heating curve was evaluated. For presentation in the graph, all curves were standardized to the sample weight.

The limit of detection of the method for the samples containing 2-methyl-2,4-pentanediol, 1,2-pentanediol, and 1,2-hexanediol was determined by examining a sample containing the alkanediol, polysorbate 60, and cetostearyl alcohol in the same ratio as in the mixed crystal. No water was added. For determining the detection limit of the samples containing 1,2-octanediol, polysorbate 60 was excluded because its melting temperature is similar to that of 1,2-octanediol. In these curves a separate melting peak of the alkanediol was expected if the amount exceeded the limit of detection.

4.3.10. Small-Angle X-ray Diffraction Measurements

X-ray measurements were performed with a small-angle X-ray diffraction system SAXSess mc² equipped with a CCD (charge-coupled device) detector (Anton Paar GmbH, Graz, Austria). A copper anode with a CuKα line wavelength of 0.154 nm

served as X-ray source. The samples were measured in a quartz capillary sample holder at 20 °C. Per measuring series 25 runs with an exposure time of 30 s were averaged.

The X-ray diffraction measurements were analyzed based on Bragg's equation

$$2\,d\,\sin\theta = n\,\lambda \qquad (1)$$

where in d represents the distance of the lattice layers of the measured crystals, θ denotes the angle of the incident X-rays referred to the planes (thus θ corresponds to half the angle between the incident and the reflected X-ray beams), λ is the wavelength of the X-ray beam, and n is a positive integer.

In a crystal, d represents the interplanar distance of lattice planes. However, in the mixed crystals examined in this study d denotes the distance between the bilayers of mixed crystals as displayed in Figure 4. Thus, in our study, d describes the lamellar spacing.

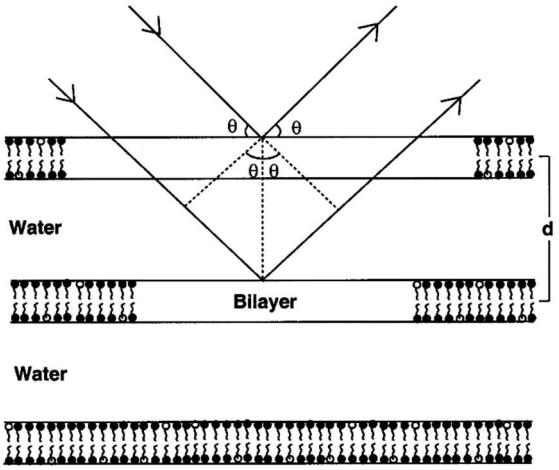

Figure 4. Schematic diagram of X-ray diffraction from bilayers reproduced with permission from G.M Eccleston, International Journal of Pharmaceutics; published by Elsevier, 2000 [13].

As can be recognized from Bragg's equation, θ is inversely proportional to the distance of the lamellar spacing as long as sinθ can be approximated by θ.

4.4. Results and Discussion

4.4.1. Influence of Alkanediols on the Storage Stability

All produced NHCs were white semisolids. Within the storage time of 6 months all formulations showed no phase separation during all six stages of the centrifugation test. Additionally, the consistency of all creams remained unchanged. This finding is

supported by the corresponding oscillation measurements (Figure 5). Figure 5 presents the storage moduli G' in the linear viscoelastic region of the prepared creams containing the different alkanediols during 6 months storage at room temperature. During the first 4 weeks, some of the creams show small changes in G' which are typical for that kind of formulation due to rearrangement of colloidal structures after intense shearing during preparation. After 1 month storage, all G' values remained nearly constant which is a clear indication that the consistency did not change essentially during storage.

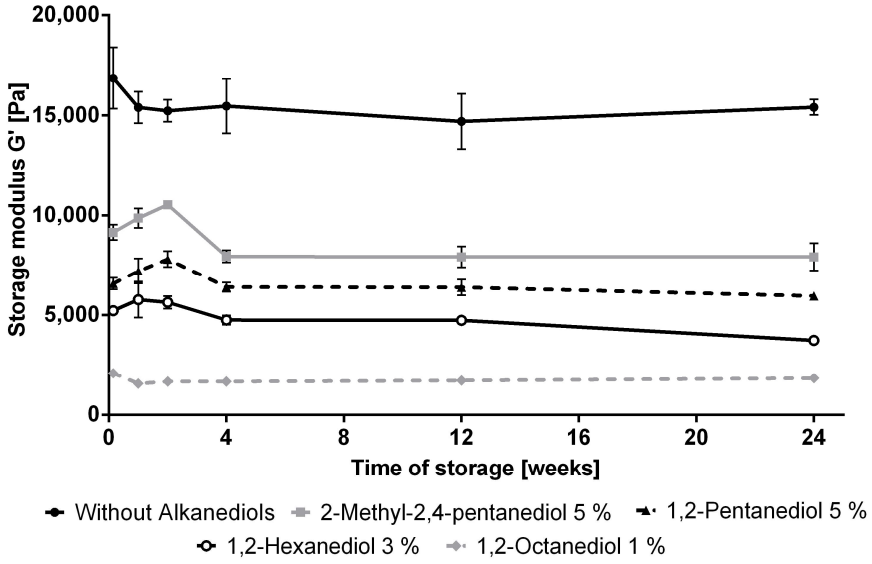

Figure 5. Influence of alkanediols on the storage modulus G', n = 3, mean ± standard deviation.

However, the consistency of the creams depends clearly on the type of the added alkanediol. Their gel strength decreases with increasing chain length of the alkanediol.

The most pronounced effect was observed for the storage modulus of the NHC supplemented with 1,2-octanediol which possesses the longest alkyl chain of the investigated alkanediols, followed by the creams containing 1,2-hexanediol and 1,2-pentanediol. 2-Methyl-2,4-pentanediol differs from this sequence. Preparations containing 2-methyl-2,4-pentanediol showed the highest storage modulus of all NHCs with the addition of alkanediols. This deviation is presumably a consequence of the different, non-vicinal positions of the hydroxy groups of this substance.

The flow points of the different creams during storage are displayed in Figure 6. The NHC without the addition of alkanediols demonstrated the highest flow point. The formulations containing 1,2-pentanediol and 2-methyl-2,4-pentanediol showed similar

flow points, whereas its value for the cream with 1,2-hexandiol was clearly reduced. 1,2-Octanediol has the highest impact on the flow point of the tested creams. It is worth to mention, that although the addition of 1,2-octanediol yielded very low flow points, the samples did not freely flow under the influence of gravity from the storage container.

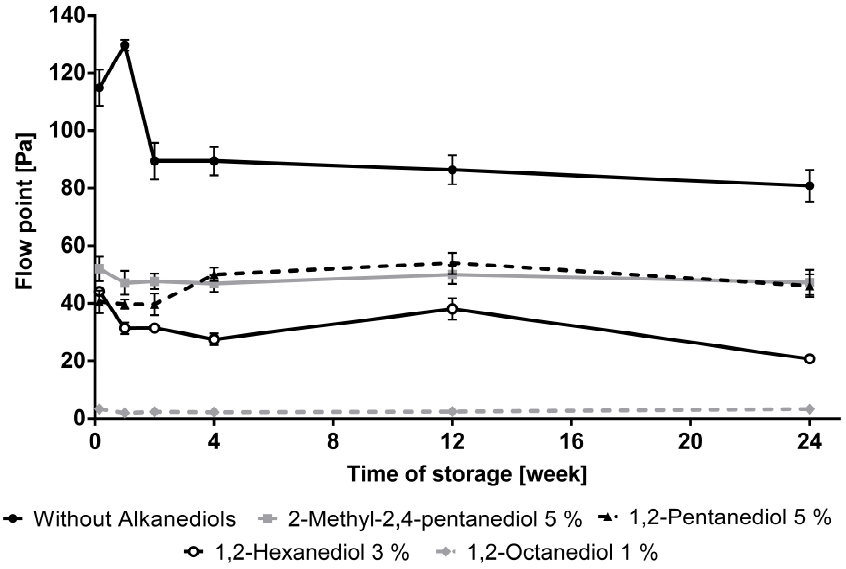

Figure 6. Influence of alkanediols on the flow point, n = 3, mean ± standard deviation.

Figure 7 represents the effect of the different alkanediols on the microscopic appearance of the NHC after 6 months storage. The pure NHC (i) and the NHC with the addition of 5% of 2-methyl-2,4-pentanediol (ii) show no visible textures. This indicates that the lamellar structures are small compared to the resolution of the polarization microscope. Further, supplementing the NHC with 1,2-pentanediol (iii) does not significantly alter the appearance compared to the pure NHC.

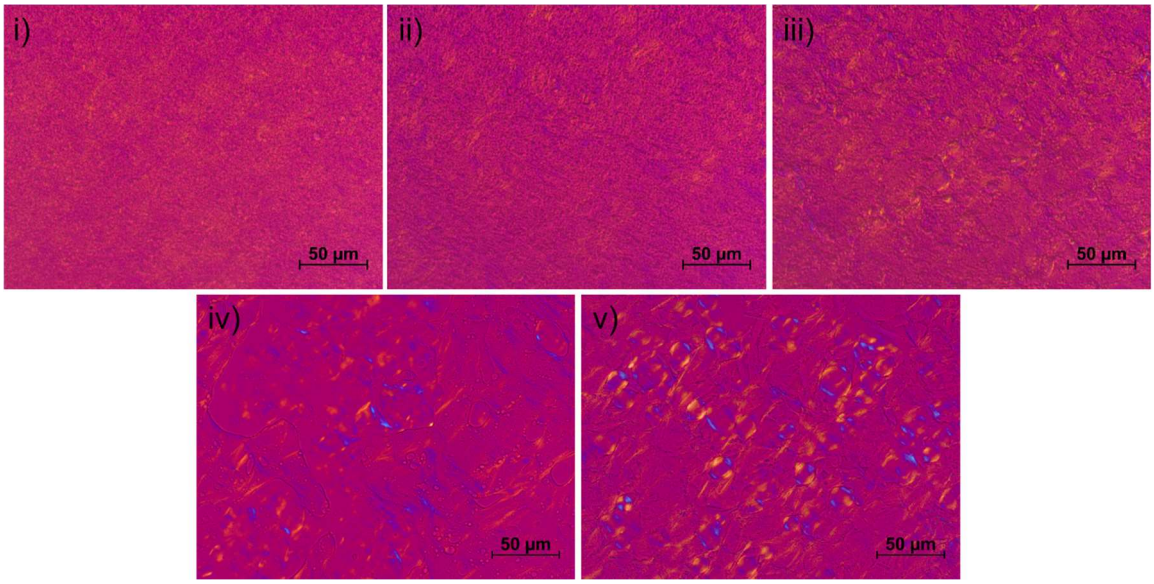

Figure 7. Polarization microscopic images of the formulations stored over 6 months: (i) pure NHC, (ii) 2-methyl-2,4-pentanediol 5%, (iii) 1,2-pentanediol 5%, (iv) 1,2-hexanediol 5%, (v) 1,2-octanediol 1%.

In contrary, the formulations with longer-chained alkanediols show clearly visible Maltese crosses indicating the presence of a lamellar phase in the cream [14]. Obviously, these longer-chain amphiphilic molecules provoked a rearrangement in the liquid crystalline structures of the creams which led to the formation of larger and thus detectable crystals.

4.4.2. Incorporation of Alkanediols into Mixed Crystals

DSC and SAXS measurements were performed to further elucidate the impact of the alkanediols on the colloidal structure of the cream which was indicated by rheometry and polarized light microscopy. To this end, mixed crystals consisting of polysorbate 60, cetostearyl alcohol, and water were prepared without and with additional alkanediols.

4.4.2.1. DSC Measurements

DSC thermograms of mixed crystals without and with 1,2-pentanediol and 2-methyl-2,4-pentanediol are shown in Figure 8. The addition of these two alkanediols (curves 2 and 3) seems to change the thermal behavior only marginally compared to plain NHC (curve 1). The melting point of the mixed crystal with additional alkanediols is slightly shifted to lower temperatures. However, it must be taken into consideration that the concentration might be too low in order to allow the detection of a separate melting

peak if present. Therefore, it cannot finally be decided from the DSC measurements whether these two alkanediols are integrated into the mixed crystal or not.

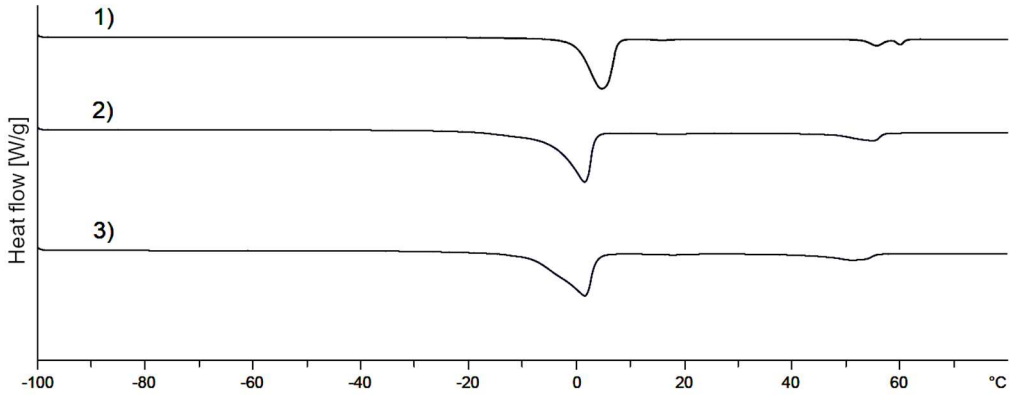

Figure 8. DSC thermograms of (1) mixed crystal without alkanediols; (2) mixed crystal: 2-methyl-2,4-pentanediol, standard concentration; (3) mixed crystal: 1,2-pentanediol, standard concentration.

The thermogram of the mixed crystal with 1,2-hexandiol shows only one melting peak as displayed in Figure 9. As the concentration of 1,2-hexanediol was clearly above its limit of detection, this finding indicates a complete incorporation of 1,2-hexanediol into the mixed crystal.

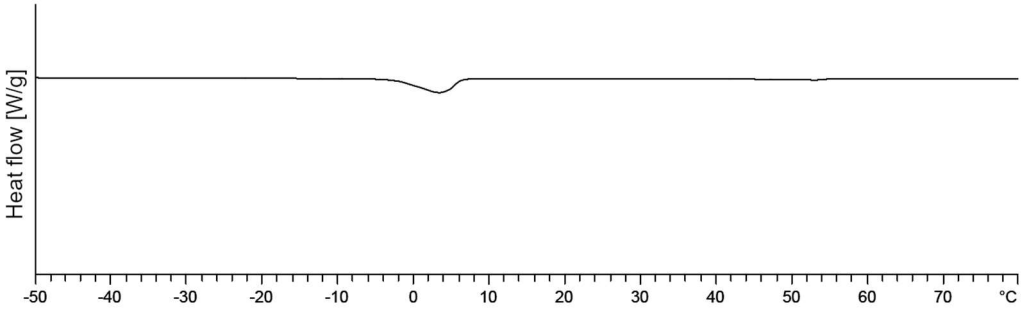

Figure 9. DSC thermogram of a mixed crystal containing 1,2-hexanediol, standard concentration.

Figure 10 depicts the thermograms of mixed crystals supplemented with 1,2-octanediol. Curves 1–3 show the melting behavior of the pure substances 1,2-octanediol (curve 1), polysorbate 60 (curve 2), and cetostearyl alcohol (curve 3). The curve of the mixed crystal with 1,2-octandiol (curve 4) shows a singular endothermic melting peak at 0.11 °C indicating a complete integration of 1,2-octanediol into the lamellar mixed crystal. In order to further challenge this system, a mixed crystal with the 10-fold amount of 1,2-octanediol was prepared. As can be seen form curve 5, also at this overdosed concentration only one endothermal event and no separate melting peak of 1,2-octandiol could be detected. This implies that 1,2-octanediol is fully

incorporated into the mixed crystal at least in the concentration range from 0 to 10% including its standard concentration of 1%.

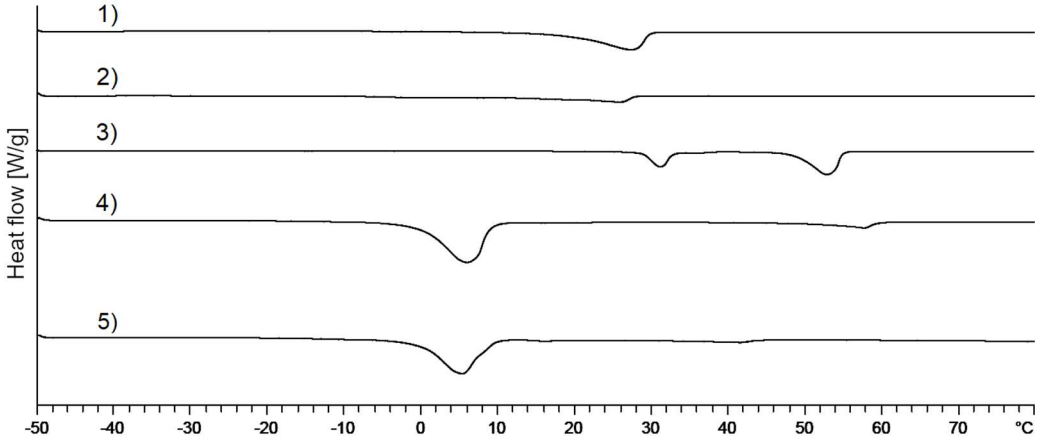

Figure 10. DSC thermograms of (1) 1,2-octanediol; (2) polysorbate 60; (3) cetostearyl alcohol; (4) mixed crystal: 1,2-octanediol, standard concentration; (5) mixed crystal: 1,2-octanediol, 10-fold concentration.

As the 1,2-octanediol concentration is further increased (Figure 11: curve 5: 20%; curve 6: 30%), the melting peaks begin to widen with increasing concentration of 1,2-octanediol. This also suggests an interaction of 1,2-octanediol with the mixed crystal leading to a less ordered structure if the content of 1,2-octanediol is augmented.

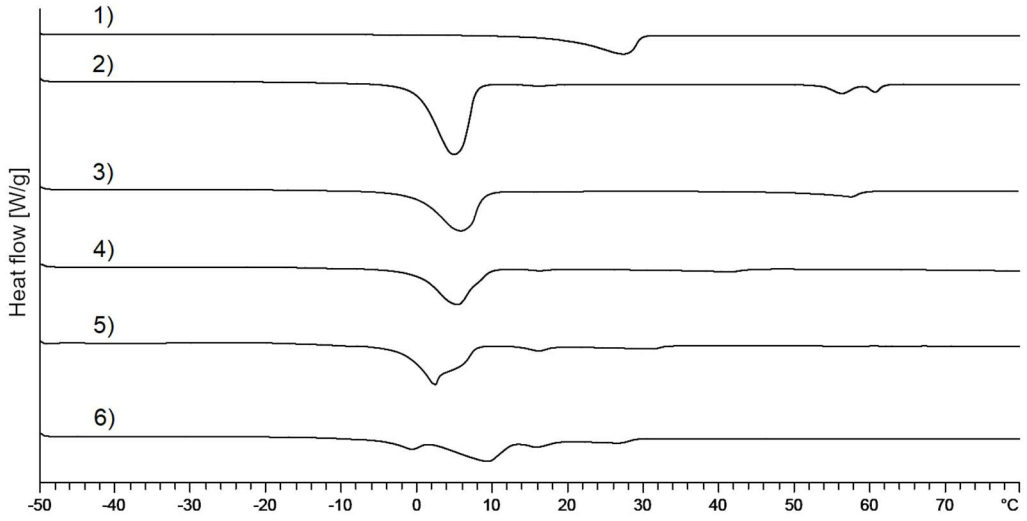

Figure 11. DSC thermograms of (1) 1,2-octanediol; (2) mixed crystal without alkanediols; (3) mixed crystal: 1,2-octanediol, standard concentration; (4) mixed crystal: 1,2-octanediol, 10-fold concentration; (5) mixed crystal: 1,2-octanediol, 20-fold concentration; (6) mixed crystal: 1,2-octandiol, 30-fold concentration.

4.4.2.2. SAXS Diffraction

In order to determine the interlamellar spacing of the various mixed crystals in the absence and presence of alkanediols, SAXS diffraction measurements were performed (Figure 12). The reflected intensity is plotted against the scattering angle 2θ, the angle between incident and diffracted X-ray beam. The corresponding lattice layer distances are summarized in Table 4. The order of magnitude of the layer lattice distances is defined by cetostearyl alcohol [13,15,16,17].

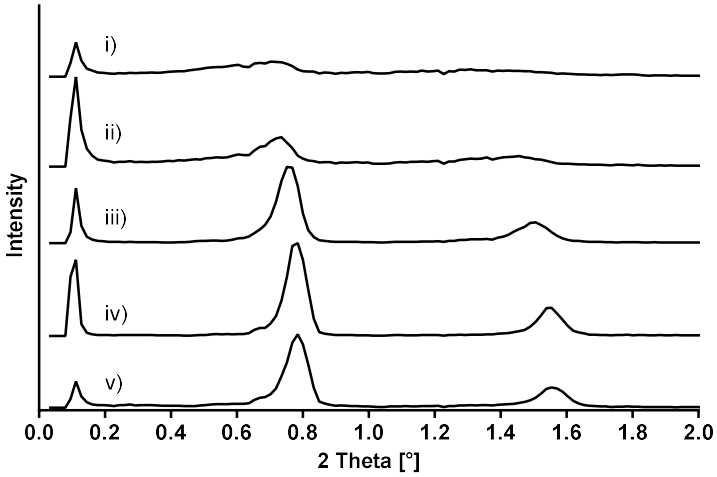

Figure 12. SAXS diffraction patterns of mixed crystals of (i) cetostearyl alcohol, polysorbate 60 and water in the presence of (ii) 2-methyl-2,4-pentanediol, (iii) 1,2-pentanediol, (iv) 1,2-hexanediol, (v) 1,2-octanediol.

Table 4. Lamellar distances of mixed crystals of cetostearyl alcohol, polysorbate 60, water and in the presence of different alkanediols.

Mixed crystals	2 θ (°)	d (nm)	Ratio
(i) in the absence of alkanediol	0.70	12.56	1.86
	1.31	6.74	
(ii) 2-methyl-2,4-pentanediol	0.74	12.00	1.98
	1.46	6.06	
(iii) 1,2-pentanediol	0.75	11.74	2.00
	1.51	5.86	
(iv) 1,2-hexanediol	0.78	11.25	1.98
	1.56	5.68	
(v) 1,2-octanediol	0.78	11.25	1.98
	1.56	5.68	

All samples show a peak in the SAXS diffraction pattern at 2θ = 0.11° (d = 78.52 nm) which represents the long-range order of the mixed crystals. In contrast, the peaks representing the near-order region are clearly affected by the addition of alkanediols. The spectrum of the mixed crystal without any alkanediol (i) shows no distinct, sharp

peak. The addition of 2-methyl-2,4-pentanediol to the mixed crystal (ii) only leads to a small change in the diffraction pattern characterized by two peaks which can be clearly identified. First-order reflections spectra of high intensity were obtained when adding 1,2-alkanediols (iii)–(v) to the mixed crystals. This is a clear indication that the 1,2-alkandiols interact with the lamellar structure of cetostearyl alcohol and polysorbate 60 provoking a rearrangement leading to more ordered and regularly organized structures [14]. This finding is consistent with the results of the polarization microscopic images which indicate a more pronounced lamellar phase when higher chained alkanediols are incorporated into the mixed crystals.

The ratio of the SAXS reflexes points directly to the lipid phase structure. If the ratio of the reflections is 1:2, 1:3, 1:4, etc., this is indicative of lamellar structures [14]. The plain cetostearyl alcohol and polysorbate 60 mixed crystals revealed a ratio of the angular distances of first and second order peaks of 1.86. This can be attributed to the fact that cetostearyl alcohol is a mixed compound which is not able to perfectly order in lamellae with a distinct spacing. The addition of alkanediols increases this ratio to values close to 2. This is not only a distinct sign of the existence of lamellar structures but also strengthens the hypothesis that the incorporation of the 1,2-alkandiol allows for a rearrangement of the fatty alcohol molecules.

Beside the peaks getting sharper and the lamellar structures getting more pronounced with addition of alkanediols, the scattering angle 2θ is shifted to higher values, as can be seen in Figure 12. The mixed crystal without an alkanediol reveals interlamellar spacings of 12.56 and 6.74 nm. Upon the addition of 1,2-alkandiols, the distance of the lattice layers of the mixed crystal decreases with increasing alkyl chain length of the 1,2-alkanediol, leading to layer distances of 11.25 and 5.68 nm in the presence of 1,2-hexandiol and 1,2-octandiol. This result suggests that the rigid crystals become more flexible upon the incorporation of the amphiphilic 1,2-alkanediols. This allows the fatty alcohol molecules to come more closely together and reduces thus the layer lattice distances.

The reduction of lamellar spacing due to a rearrangement of the lipids can be a hint that less water is enclosed between the layers of the mixed crystal [13]. This shifts the ratio between interlamellarly fixed water and bulk water in direction of the bulk water. Hence, the gel structure of the nonionic cream has to immobilize a larger amount of

bulk water [18]. If this is not possible, the reorganization of liquid crystalline and crystalline phases reduces the ability of the cream to immobilize water making the formulations less stable.

In contrast, 2-methyl-2,4-pentanediol showed only a small impact on the spacing of the layers leading to distances of 12.00 and 6.06 nm, respectively. Compared to the 1,2-alkanediols, the incorporation of 2-methyl-2,4-pentanediol into the mixed crystal is less favorable because it has the hydroxyl groups on the positions C2 and C4. This reduces its amphiphilicity and consequently, both, the peak intensity and the layer distance resemble the plain mixed crystal.

4.4.3. Efficacy of Antimicrobial Preservation

To evaluate the preservative activity of the different alkanediols, a test for efficacy of antimicrobial preservation was performed. The results were evaluated according to the acceptance criteria specified in the Ph. Eur. [12]. According to Ph. Eur., the $\log_{10}$ reduction in the number of viable micro-organisms against the value obtained for the inoculum serves as the basis for the evaluation. Criterion A represents the usually required efficacy. If, in justified cases, criterion A cannot be met, e.g., if there is an increased risk of adverse effects, criterion B must be attained.

Table 5 summarizes the results of the test for efficacy of antimicrobial preservation. NHC conventionally preserved with 0.1% sorbic acid (is) served as a positive control. Formulations containing 5% 2-methyl-2,4-pentanediol (ii), 5% 1,2-pentanediol (iii), 3% 1,2-hexanediol (iv), and 1% 1,2-octanediol (v) were tested. For the creams with shorter chained alkanediols, formulations were analyzed containing additionally 0.05% sorbic acid, which is half the concentration typically used in dermal formulations (Table 5: formulations (iis) and (iiis)). These creams were tested to investigate whether the concentration of a commonly used preservative can be reduced in case the alkanediol alone does not have a sufficient preservative effect.

Table 5. Acceptance criteria according to the test for efficacy of antimicrobial preservation Ph. Eur. 5.1.3. achieved by the formulations: (is) NHC conventionally preserved with 0.1% sorbic acid, (ii) 5% 2-methyl-2,4-pentanediol, (iis) 5% 2-methyl-2,4-pentanediol + 0.05% sorbic acid, (iii) 5% 1,2-pentanediol, (iiis) 5% 1,2-pentanediol + 0.05% sorbic acid, (iv) 3% 1,2-hexanediol, (v) 1% 1,2-octanediol.

Formulation	(is)	(ii)	(iis)	(iii)	(iiis)	(iv)	(v)
Staph. aureus	A	A	A	A	A	A	A
Ps. aeruginosa	A	A	A	A	A	A	A
E. coli	A	A	A	A	A	A	A
C. albicans	A	A	A	A	A	A	A
Asp. brasiliensis	A	-	A	B	A	A	A

The formulations containing the recommended amount of 1,2-hexanediol (iv) and 1,2-octanediol (v) met the “A” criterion according to Ph. Eur. for all tested strains. This underlines that the increasing amphiphilicity of these compounds increases their antimicrobial activity even at the lower use concentrations.

On the contrary, the amphiphilic character of 1,2-pentanediol is less pronounced leading to partially impaired antimicrobial activity. Consequently,1,2-pentandiol achieved only criterion B for *Asp. brasiliensis*. However, this weak effect against the mold could be compensated by supplementing the cream with 0.05% sorbic acid.

The lowest antimicrobial activity was found for 2-methyl-2,4-pentanediol which failed both Ph. Eur. criteria for *Asp. brasiliensis*. As already discussed above, it is supposed that this is a consequence of the location of its hydroxy groups in positions 2 and 4 reducing the amphiphilicity and thus making an integration into the lipid bilayer of the cell membrane of the microorganism less favorable. However, even in this case supplementing the cream with 0.05% sorbic acid was sufficient to attain the “A” criterion for *Asp. brasiliensis*. Although it is not sufficiently effective on its own, 2-methyl-2,4-pentanediol can therefore be used to reduce the concentration of the commonly used preservative sorbic acid.

These results are completely in line with the proposed mechanism of antimicrobial activity of the alkanediols which is attributed to their amphiphilic structure. Hence, these substances can penetrate the lipid bilayer of the microbial cell membrane. As a result, substrate transport is inhibited, and vital cellular components leak. The disruptive effect increases as a consequence of the deeper penetration into the bilayer as a function of the chain length [19]. The surface activity of the alkanediols increases

with increasing chain length of the alkyl chain (data not shown)[1]. 2-Methyl-2,4-pentanediol differs from this sequence due to its non-vicinal hydroxy groups. Consequently, its interfacial activity and its antimicrobial activity are markedly reduced.

4.5. Conclusions

In the present study, alkanediols were investigated as alternative preservatives for the nonionic hydrophilic cream DAB as a typical representative of a nonionic cream. The antimicrobial activity of these compounds is linked to their amphiphilic structure. The latter could be easily demonstrated by measuring the interfacial activity of these compounds. Their amphiphilic character enables on the one hand the interaction with cell membranes of microorganisms but allows on the other hand also to affect the colloidal structure of creams. The present study revealed that alkanediols change the colloidal structure of the NHC as a consequence of the incorporation of these amphiphilic substances into the mixed crystal of the cream. The detected decrease of the lamellar distances of the mixed crystal results in a diminished consistency of the creams. The observed reduction of the storage modulus G' and the flow point was in line with the chain length of the added alkanediol and came along with an increasingly ordered mixed crystal which is responsible for the semi-solid behavior of the cream. The lamellar nature manifests in characteristic "Maltese cross" textures which become visible under a polarized microscope when the NHC is supplemented with 1,2-hexanediol or 1,2-octanediol. This means, the stability of the NHC is reduced by the incorporation of an alkanediol in its mixed crystal. However, as no visible phase separation was detected even during an extended centrifugation test, it can be concluded that the changes in the colloidal structure did practically not affect the ability of the lamellar gel network to immobilize the incorporated water.

Furthermore, alkanediols proved to be excellent alternatives for the antimicrobial preservation of the NHC, wherein the preservative activity raises with their alkyl chain length and could be directly linked to their amphiphilicity. All formulations containing 1,2-alkanediols attained mostly criterion "A" in the test for efficacy of antimicrobial preservation. 1,2-octanediol proved to have the highest activity, whereas 1,2-pentanediol was less efficient and achieved only criterion B for the test mold.

[1] See Chapter 3, Interfacial Activity of the Alkanediols

The only alkanediol, that differs largely from the others in all addressed properties was 2-methyl-2,4-pentanediol which differs in its chemical structure from the other alkanediols as it has no vicinal hydroxy groups. It showed the lowest interfacial activity and had the lowest impact on both the stability and the preservation of the NHC. Its integration into the lamellar lipid structure of the cell membranes as well as the mixed crystal of the NHC is sterically hindered.

In general, it can be concluded, that 1,2-alkanediols can be used as effective alternative preservatives in dermal formulations. Changes in the consistency of the preparation ultimately limit the use of 1,2-alkanediols. Besides, ongoing studies are aiming to clarify if such an alternative preservation affects release and skin penetration of drug substances.

Author Contributions

Conceptualization, M.S. and R.D.; methodology, M.S. and R.D.; investigation, M.S.; data curation, M.S.; writing—original draft preparation, M.S.; writing—review and editing, R.D.; visualization: M.S.; supervision: R.D.; project administration: R.D. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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5. The Effect of Alkanediols on the Release of Triamcinolone Acetonide from Semisolid Dosage Forms

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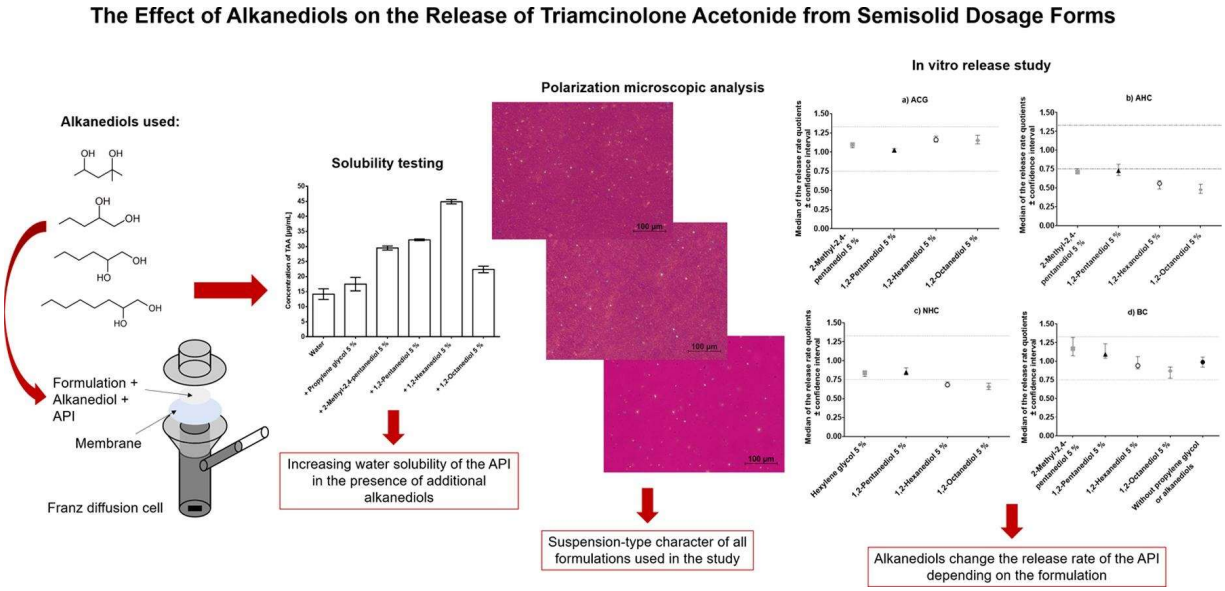
Graphical abstract:



5.1. Abstract

Alkanediols are frequently used as alternative antimicrobial preservatives for dermal formulations. However, these substances can also have an influence on the biopharmaceutical properties of the applied preparations. Therefore, the influence 2-methyl-2,4-pentanediol, 1,2-pentanediol, 1,2-hexanediol and 1,2-octanediol on the release of triamcinolone acetonide (TAA) from four different commonly used semi-solid vehicles was investigated. In addition, the solubility of TAA in aqueous alkanediol

solutions was evaluated. It was observed that its solubility increases as a function of chain length of the alkanediol, with exception of 1,2-octanediol. This can be related to the corresponding solubility parameters. Despite alkanediols increase the aqueous solubility of TAA, polarization microscopic images revealed that a significant amount of the drug is present in the suspended state in all formulations. Therefore, TAA release was proportional to the square root of time, indicating Higuchi kinetic. Alkanediols modify the release of TAA depending on the used base. The addition of alkanediols to the hydrogel formulation result in a slightly augmented release rate of the drug with increasing chain length of the added alkanediol. In contrast, alkanediols having longer chain lengths diminish the TAA release rate from all tested creams. Consequently, TAA release revealed to be partially inequivalent upon the addition of alkanediols.

Keywords: Alkanediols; Preservation; In vitro release; Triamcinolone acetonide; Solubility enhancement

5.2. Introduction

A large number of commonly used antimicrobial preservatives in dermal preparation are increasingly under debate, especially when being used in pediatric formulations (Committee for Medicinal Products for Human Use and European Medicines Agency, 2014). Mainly, some preservatives, particularly parabens, are under discussion because of their endocrine disrupting properties (Darbre and Harvey, 2008; Matwiejczuk et al., 2020). Thus, there is a rising demand for alternative compounds for antimicrobial preservation which are non-hazardous to health as well as meet microbiological quality acceptance criteria for cutaneous preparations. Alkanediols could be used as substitutes for conventional preservatives. The chemical structures of the alkanediols used in this study are presented in Fig. 1.

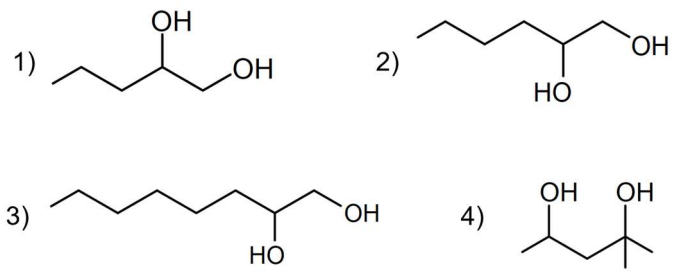


Fig. 1. Chemical structures of the alkanediols used: (1) 1,2-pentanediol, (2) 1,2-hexanediol, (3) 1,2-octanediol, (4) 2-methyl-2,4-pentanediol.

1,2-Pentanediol, 1,2-hexanediol and 1,2-octanediol are 1,2-alkanediols that have vicinal hydroxy groups at positions C1 and C2. However, 2-methyl-2,4-pentanediol (hexylene glycol) deviates from this basic structure since it has two hydroxy groups at positions C2 and C4. The preservative effect of the above listed alkanediols is attributed to their amphiphilic properties, which enable them to penetrate into the cell membranes of microorganisms (Okukawa et al., 2019). As a result of their amphiphilic structure, alkanediols also interact with the formulation itself (Pillai et al., 2013). In addition, these substances exhibit moisturizing and solubilizing properties and are therefore used as multifunctional excipients in cosmetic formulations (Ashland LLC, 2017; Schrader, 1995). Moreover, alkanediols act as penetration enhancers (Duracher et al., 2009; Heuschkel et al., 2008). The Cosmetic Ingredient Review Expert Panel evaluates the use of the alkanediols investigated in our studies as safe when they are applied in the standard concentrations (Bergfeld et al., 2011; Liebert, 1985).

In a previous study (Sigg and Daniels, 2020) the effect of alkanediols on the physical stability of the nonionic hydrophilic cream (NHC) according to the German Pharmacopeia (DAB) (Deutsche Arzneibuch-Kommission, 2020) was investigated. Alkanediols were found to incorporate into the lamellar mixed crystal structures of the NHC with an increasing effect with raising alkyl chain length of the added alkanediol. Consequently, consistency and stability of the cream are slightly impaired. Moreover, antimicrobial activity increased with the alkyl chain length. The test of efficacy of antimicrobial preservation was passed for the cream containing 5% 1,2-pentanediol. 3% 1,2-hexanediol were also effective enough to have an antimicrobial effect. In case of 1,2-octanediol, even a 1% concentration was sufficient for an adequate preservation of the tested NHC. On the contrary, 2-methyl-2,4-pentanediol in 5% concentration was not sufficient to meet the test requirements. However, this substance could be used to reduce the required amount of a conventional preservative. The observed effects can be directly attributed to the amphiphilicity of the tested alkanediols. Hence, it can be argued, that the addition of alkanediols as alternative antimicrobial preservatives might also affect the biopharmaceutical properties of a dermal preparation.

Therefore, in the present study, we focused on the influence of alkanediols on the release of an active pharmaceutical ingredient (API) from four different commonly used semi-solid vehicles. For this purpose, we selected the NHC and the anionic hydrophilic cream (AHC) which have a comparable colloidal structure but differ in the charge of

the hydrophilic part of the emulsifier (Junginger, 1984). The basic cream (BC) according to the German Drug Codex DAC (DAC) (Bundesvereinigung Deutscher Apothekerverbände ABDA, 2020) was selected due to its so called amphiphilic character which is characterized by a tricoherent structure (Junginger, 1992). The aqueous carbomer gel (ACG) according to the DAB was chosen as a classical example for a hydrogel base.

Triamcinolone acetonide (TAA) was selected as the model drug due to its wide spread use in the treatment of inflammatory, allergic and pruritic dermatoses (Bracher et al., 2020; Wohlrab et al., 2017). Like most corticosteroids, TAA has only a limited solubility in commonly used vehicles for cutaneous application.

Mandatory prerequisite for drug transport into the skin are the dissolution within and the release from the vehicle (Kalia and Guy, 2001). Therefore, it was the purpose of the present study to examine the impact of added alkanediols on the TAA solubility and release and more specifically, to investigate whether the effect is related to the composition and structure of the vehicle.

5.3. Material and Methods

5.3.1. Material

1,2-Pentanediol, 2-methyl-2,4-pentanediol, and 1,2-hexanediol (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany), polysorbate 60 (Kolliphor PS 60), cetostearyl alcohol (Kolliwax CSA), cetyl alcohol (Kolliwax CA), emulsifying cetostearyl alcohol type A (Kolliphor CS A) (BASF SE, Ludwigshafen, Germany), glycerol 85%, propylene glycol (Dr. Willmar Schwabe GmbH & Co. KG, Karlsruhe, Germany), white soft paraffin (Sasol Performance Chemicals, Hamburg, Germany), liquid paraffin (Hansen & Rosenthal KG, Hamburg, Germany), carbomer 50.000 (Fagron GmbH & Co. KG, Glinde, Germany), sodium hydroxide (Chemical supply of the University, Tuebingen, Germany), medium-chain triglycerides (Evonik Industries AG, Essen, Germany), glycerol monostearate 60, macrogol 20 glycerol monostearate, disodium hydrogen phosphate, potassium dihydrogen phosphate, sodium chloride, triamcinolone acetonide micronised (Caesar & Loretz GmbH, Hilden, Germany), bovine serum albumin (Carl Roth GmbH & Co. KG, Karlsruhe, Germany), acetonitril HPLC gradient grade (Honeywell Specialty Chemicals Seelze GmbH, Seelze, Germany), PTFE filters

(pore size: 0.2 µm, diameter: 25 mm, Chromafil Xtra H-PTFE-20/25, Macherey-Nagel GmbH & Co. KG, Dueren, Germany), polycarbonate membrane (pore size: 0.4 µm, diameter: 25 mm, Whatman Nuclepore Track-Etched Membranes, Global Life Sciences Solutions USA LLC, Marlborough, United States). 1,2-Octanediol (dermosoft Octiol) was kindly donated by Evonik Dr. Straetmans GmbH, Hamburg, Germany.

5.3.2. Preparation of test formulations

Table 1. Composition (in % w/w) of the pure formulations without TAA and alkanediols.

(a) ACG		(b) AHC		(c) NHC		(d) BC	
Carbomer 50.000	0.5	Emulsifying cetostearyl alcohol type a	9.0	Polysorbate 60	5.0	Glycerol mono-stearate 60	4.0
Sodium hydroxide solution 5%	3.0	Liquid paraffin	10.5	Cetostearyl alcohol	10.0	Cetyl alcohol	6.0
Purified water	96.5	White soft paraffin	10.5	White soft paraffin	25.0	Medium-chain triglycerides	7.5
		Purified water	70.0	Glycerol 85%	10.0	White soft paraffin	25.5
				Purified water	50.0	Macrogol 20 glycerol monostearate	7.0
						Propylene glycol	10.0
						Purified water	40.0

The composition of the compendial formulations used in this study are summarized in Table 1. Test formulations were prepared from these bases by adding 0.1% TAA, which corresponds to its upper recommended use concentration (Bracher et al., 2020). Further, all formulations were prepared with the addition of 5% of each alkanediol, subtracting the amount of alkanediol added from the amount of water.

For preparation of the ACG, carbomer and TAA were weighted into an Unguator jar. After adding either water or the water-alkanediol mixture and sodium hydroxide solution, the “gel” programme of an Unguator 2100 mixing system (Gako International GmbH, Scheßlitz, Germany) was applied.

To prepare the AHC, emulsifying cetostearyl alcohol type A, liquid paraffin and white soft paraffin were melted in an Unguator jar in a water bath at 70 °C. Water was separately heated to the same temperature. If required, the alkanediol was added to the warmed water. Both phases were mixed by using the Unguator 2100 mixing system with a cycle of 1 min fast stirring (2000 rpm (revolutions per minute) mixing motor, 2200 rpm lifting motor) and 10 min slow stirring (750 rpm mixing motor, 800 rpm lifting motor). These two steps were repeated two times.

The NHC and the BC were both produced the same way. After heating the lipophilic and hydrophilic phases separately to 70 °C, the phases were manually pre-mixed and finally homogenized using the Unguator 2100 mixing system with the programme “normal”. The standard BC contains 10% propylene glycol which is also an alkanediol. Therefore, 10% propylene glycol was substituted for 5% of the other alkanediols in order to separate the effect of the alkanediols. The deficient 5% were replaced by water. To investigate the influence of propylene glycol, the BC was also prepared without both propylene glycol and alkanediol, with the missing amount completely substituted by water.

TAA was added to the preformed bases using the “suspension < 2%” programme of the Unguator 2100 mixing system.

5.3.3. Polarized light microscopy

Undiluted samples were thinly spread on a glass microscope slide and covered with a coverslip. An Axio Imager Z1 microscope (Carl Zeiss, Jena, Germany) with crossed polarizers and a ¼ λ-plate was used to capture the polarization microscopic images. The magnification of the microscope was 20-fold.

5.3.4. Saturation solubility of TAA

To measure the saturation solubility of TAA in aqueous media in absence and presence of 5% of the different alkanediols an excessive amount of TAA (approx. 15 mg) was weighed into glass vials. Subsequently 3 mL of the aqueous phase was added. The media were magnetically stirred in the crimped vials under light protection at 1,000 rpm for 72 h at room temperature. Thereafter, the samples were centrifuged for 30 min at 6,000 rpm (Megafuge 1.0 R, Heraeus Holding GmbH, Hanau, Germany). This led, on the one hand, to the separation of crystalline TAA. On the other hand, in the case of 1,2-octanediol, which is not completely miscible with water, this resulted in a complete phase separation of an aqueous and an 1,2-octanediol phase in equilibrium with excess TAA. Subsequently, the aqueous supernatant was filtered through a 0.2 µm PTFE filter (Chromafil Xtra H-PTFE-20/25, Macherey-Nagel, Dueren, Germany). Prior to the determination of the concentration by high performance liquid chromatography (HPLC), one part ethanol was added to avoid re-precipitation of TAA in the aqueous solutions. Each experiment was performed in triplicate.

5.3.5. Solubility parameters

The Hildebrand solubility parameter δ of a substance describes the attractive strength between molecules of the material and is defined as the square root of the cohesive energy density, expressed by Eq. (1)

$$\delta = \sqrt{\frac{\Delta E_v}{V_m}} \quad (1)$$

where ΔE_v stands for the energy of vaporization and V_m for the molar volume of the substance (Brandrup et al., 1990; Hildebrand and Scott, 1950).

The solubility parameters of substances can be calculated according to Fedors (Fedors, 1974) group contribution method using Eq. (2)

$$\delta = \sqrt{\frac{\sum \Delta e_i}{\sum \Delta v_i}} \quad (2)$$

with Δe_i and Δv_i, respectively denote the additive atomic and group contributions to the energy of vaporization and the molar volume.

For mixtures, the solubility parameter of each solvent mixture δ_s is composed according to Eq. (3) (Squillante et al., 1997)

$$\delta_s = \sum \delta_i \cdot x_i \quad (3)$$

wherein δ_i is the solubility parameter of the respective component, and x_i is its proportion in the mixture.

The difference of the solubility parameters Δδ of TAA (δ_{TAA}) and the solvent mixture (δ_s) was calculated according to Eq. (4).

$$\Delta\delta = \delta_s - \delta_{TAA} \quad (4)$$

These differences (Δδ) were compared to the TAA saturation solubility in the respective water-alkanediol mixtures.

5.3.6. In vitro release experiments using Franz diffusion cells

Release experiments were carried out using modified Franz diffusion cells (Gauer Glas, Puettlingen, Germany) with a receptor-volume of 12 mL and an inner diameter of 15 mm corresponding to an effective release area of 1.77 cm^2. Since TAA is a

lipophilic drug, the solubility in pure PBS is not sufficient to achieve sink conditions in the following experiments. The saturation solubility of TAA in PBS was found to be 14.72 ± 1.02 µg/mL. The use of a biorelevant dissolution medium is useful in product development and for establishing of vitro-in vivo correlations (Baert et al., 2009; Scientific Committee on Consumer Safety, 2010; Shah, 2005). BSA containing receptor media are recommended for transdermal in vitro studies, as BSA has a high binding capacity for lipophilic drugs as TAA and thus increases their solubility (Cross et al., 2003). Because of these considerations, BSA was added to PBS at a physiological concentration of 4% (Barbosa et al., 2010), which increased the saturation solubility of TAA more than tenfold to 181.54 ± 16.59 µg/mL. For this reason, PBS with the addition of 4% BSA was used as release medium for the following release experiments. It was warmed to 32 °C and stirred at a speed of 500 rpm. A hydrophilic polycarbonate membrane (Global Life Sciences Solutions USA LLC, Marlborough, United States) with a pore diameter of 0.4 µm was employed to separate donor and receptor compartment. To work under infinite dosing conditions (Food and Drug Administration, 1997), 1 g of the test formulation was applied onto the membrane of the donor compartment. The donor chamber was covered with Parafilm to avoid evaporation. After 0.5 h, 1 h, 2 h, 4 h, 6 h, 8 h, 10 h 0.5 mL of the receptor phase was removed and substituted by 0.5 mL of fresh, prewarmed receptor medium. The samples were further processed as described below. Studies were performed in triplicate.

5.3.7. Sample preparation and TAA assay

Prior to the HPLC analysis and if necessary, BSA was precipitated by adding three parts acetonitrile to one part of the sample (Kong, 2005). After vortexing for 10 s (Vortex 2, IKA-Werke GmbH & Co. KG, Staufen, Germany), the samples were centrifugated for 15 min at 13,400 rpm (MiniSpin, Eppendorf AG, Hamburg Germany) to pellet BSA. The clear supernatant was analysed by means of an HPLC system (LC-20AT, DGU-20A5R, SIL-20AC HAT, CTO-10ASVP, CBM 20-A, Shimadzu GmbH, Duisburg, Germany) equipped with a UV detector (SPD 20A) to quantify TAA. The separation was performed at 30 °C using an RP-18 column (Nucleosil 100-5 C18 125/4, Macherey-Nagel GmbH & Co. KG, Dueren, Germany) as the stationary phase and acetonitrile–water (40:60, v:v) as the mobile phase at a flow rate of 0.8 mL/min. A sample volume of 20 µL was injected and the UV-absorbance was determined at a

wavelength of 254 nm. The retention time of TAA was 4.8 min. The limit of quantification (LOQ) of the method for the release experiment samples was 0.24 µg/mL calculated according to the ICH (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use) guidelines (The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, 2005).

5.3.8. Equivalence test

The cumulative amount of TAA released per unit area [µg/cm^2] was calculated from the concentration in the receptor medium at each of the above indicated sampling points and plotted as a function of time. In addition, the cumulative amount released was also plotted against the square root of time. This representation should result in a straight line wherein the TAA release rate can be derived from the slope (Food and Drug Administration, 1997). A modified method for equivalence testing from the SUPAC-SS guideline of the FDA (Food and Drug Administration) (Food and Drug Administration, 1997) was used in order to assess the influence of the addition of the different alkanediols on the release of TAA. Each set of experiments comprised the reference formulation and the corresponding test formulations containing the different alkanediols. For the equivalent test, the ratios of the release rates of the test formulations (T) to the release rate of the reference (R) were calculated. Since each experiment was performed in triplicate, nine different T/R ratios were obtained for each single test formulation. In the following step the median as well as the confidence interval (CI) of the median of these nine ratios were calculated according Eqs. (5) and (6) (Hedderich and Sachs, 2018)

$$x_{(h)} < x < x_{(n-h+1)} \quad (5)$$

$$h = \frac{(n - 1 - z \cdot \sqrt{n})}{2} \quad (6)$$

wherein x stands for the median, n representing the number of T/R ratios and z = 1.645 for 90% CI. Consequently, the second and eighth value of the nine T/R ratios form the confidence interval. If the 90% confidence interval falls within the limits of 75% and 133.33%, it is supposed that the release rate of the test formulation is equivalent to the release rate of the reference. Otherwise, it is assumed that the release rates of the formulations are not equivalent.

5.4. Results and discussion

5.4.1. Solubility of TAA in the formulations

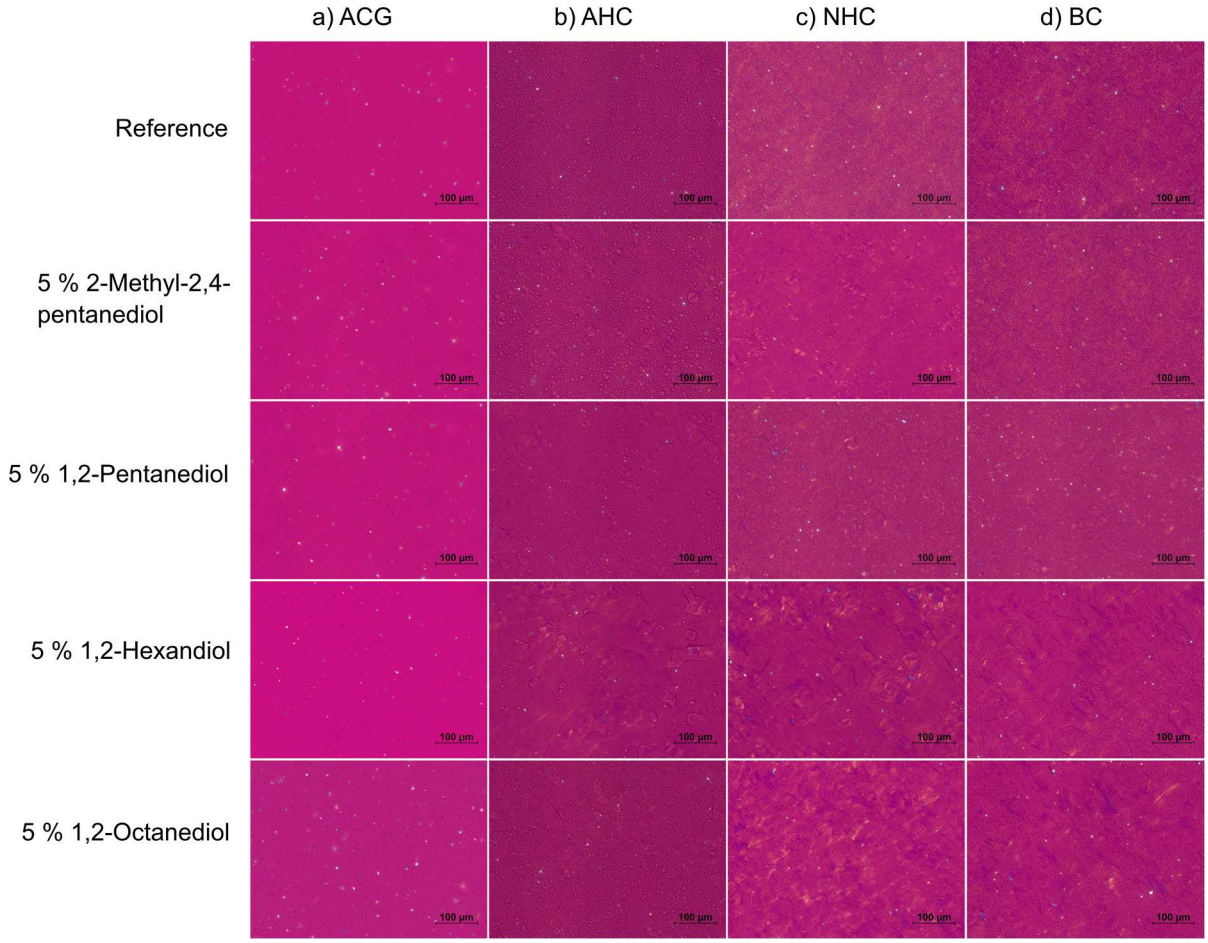


Fig. 2. Polarization microscopic images of the tested formulations containing 0.1% TAA.

Fig. 2 shows polarized light micrographs of all tested formulations containing 0.1% TAA. The first row depicts the reference formulations without additional alkanediol. The following rows depict samples with 5% of 2-methyl-2,4-pentanediol, 1,2-pentanediol, 1,2-hexanediol, and 1,2-octanediol, respectively. The small crystals that are present in all images can be assigned to be TAA which is homogeneously distributed in all formulations. In the creams, TAA was predominantly associated with the lipid phase and the mixed crystal phase of the hydrophilic emulsifier and cetostearyl alcohol or cetyl alcohol and/or glycerol monostearate. Obviously, 5% of the tested alkanediols are not sufficient to completely dissolve TAA.

5.4.2. Saturation solubility of TAA in water alkanediol mixtures

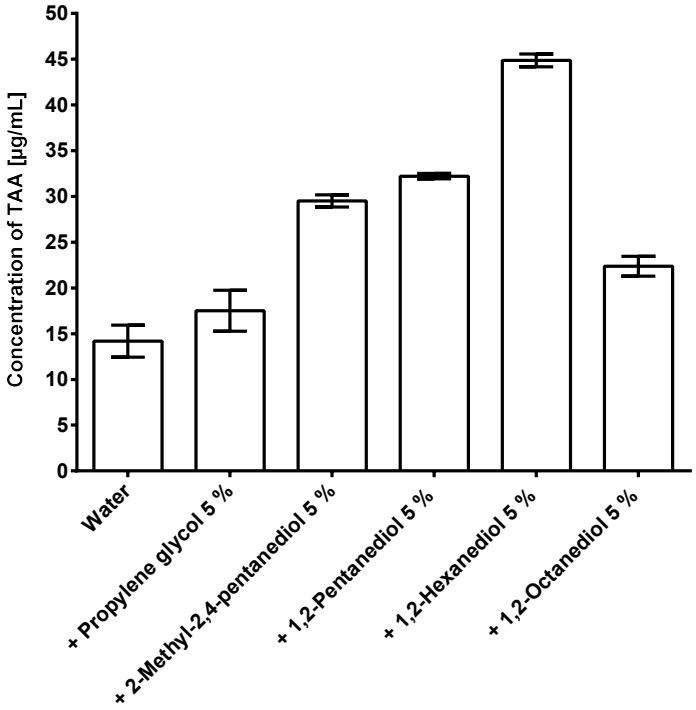


Fig. 3. Saturation solubility of TAA in different mixtures of water + 5% alkanediol, n = 3, mean ± standard deviation.

The influence of the alkanediols on the saturation solubility of TAA is displayed in Fig. 3. The solubility of TAA in purified water was determined to be 14.19 ± 1.76 µg/mL, which is comparable to its solubility in PBS. The solubility of the drug significantly rises with addition of the alkanediols. Moreover, it can clearly be observed that with increasing chain length of the added alkanediol the solubility of TAA is enhanced. The polarity of the alkanediols decreases as a function of the chain length, resulting in an improved solubility of the lipophilic TAA. The highest solubility is obtained when 5% 1,2-hexanediol are added to water, yielding a saturation solubility of 44.86 ± 0.71 µg/mL, which is more than three times the solubility of TAA in water. The solubility does not increase when the chain length is further increased. This finding is related to the limited water solubility of 1,2-octanediol (18.2 mg/ml (https://go.drugbank.com/drugs/DB14589)) in comparison to the shorter-chained alkanediols due to its too lipophilic character (Alany et al., 2000). Consequently, the TAA solubility is only improved to a limited extend. The results of the saturation solubility measurements are compared with the solubility parameters δ for these substances which allow to predict theoretically the TAA solubility in the alkanediols. The solubility parameters of TAA, water and propylene glycol were taken from literature

(Abou-ElNour et al., 2019; Burke, 1984). The respective solubility parameters of the alkanediols were calculated according to Fedors group contribution method (Fedors, 1974) by means of Eq. (2). Results are summarized in Table 2. A low δ-value of a solvent correlates to a high affinity for lipophilic substances. Moreover, the smaller the difference of the δ-values between the API and the solvent, the higher the solubility of the API in the corresponding solvent (Suzuki and Sunada, 1998). Accordingly, the solubility of TAA increases when the solubility parameter of the solvent approaches 20.1 $(MPa)^{½}$ and Δδ converges to zero.

Table 2. Solubility parameters (δ) of the substances, solubility parameters of 5% alkanediol solution ($δ_s$) and differences of solubility parameters between the solvent mixture and TAA (Δδ) compared to the TAA solubility in aqueous 5% alkanediol solutions.

Substance	δ $[(MPa)^{½}]$	$δ_s$ $[(MPa)^{½}]$	Δδ $[(MPa)^{½}]$	Solubility of TAA in a 5 % solution [µg/mL]
TAA	20.10 [a]	-	-	-
Water	48.00 [b]	48.00	27.90	14.19 ± 1.76
Propylene glycol	30.70 [b]	47.14	27.04	17.51 ± 2.24
2-Methyl-2,4-pentanediol	26.79	46.94	26.84	29.52 ± 0.66
1,2-Pentanediol	24.98	46.85	26.75	32.22 ± 0.28
1,2-Hexanediol	24.13	46.81	26.71	44.86 ± 0.71
1,2-Octanediol	22.92	47.54*	27.44	22.40 ± 1.08

[a] Abou-ElNour et al. (2019).
[b] Burke (1984).
* Calculation based on a solubility of 1.82%.

The calculated Δδ-values are in line with the results of the solubility measurements. The Δδ-values of the water-alkanediol mixtures decrease with increasing chain length of the alkanediol. Correspondingly, the TAA solubility increases. However, 1,2-octanediol deviates from this sequence. As discussed above, this is a consequence of the limited miscibility of 1,2-octanediol and water. A solvent for which the Δδ-value is equal to or less than 2 $(MPa)^{½}$ is regarded as a good solvent for a respective API. For Δδ-values greater than 2 $(MPa)^{½}$, the solvent is considered to be a nonsolvent (Venkatram et al., 2019). Based on this definition, all tested water-alkanediol mixtures are nonsolvents, since all Δδ-values are in the range of 26–28 $(MPa)^{½}$. With a solubility of less than 0.01%, a substance is considered practically insoluble in a particular solvent according to the European Pharmacopoeia (Ph. Eur.) (European Pharmacopoeia Ph. Eur., 2020). In our study, the TAA solubility is the highest for the water-hexanediol mixture with a numerical value of approximately 45 µg/mL, which corresponds to 0.0045%. Thus, although the solubility of TAA

significantly increases with the addition of alkanediols, TAA has to be referred to be practically insoluble in all tested solvent mixtures.

5.4.3. Release studies

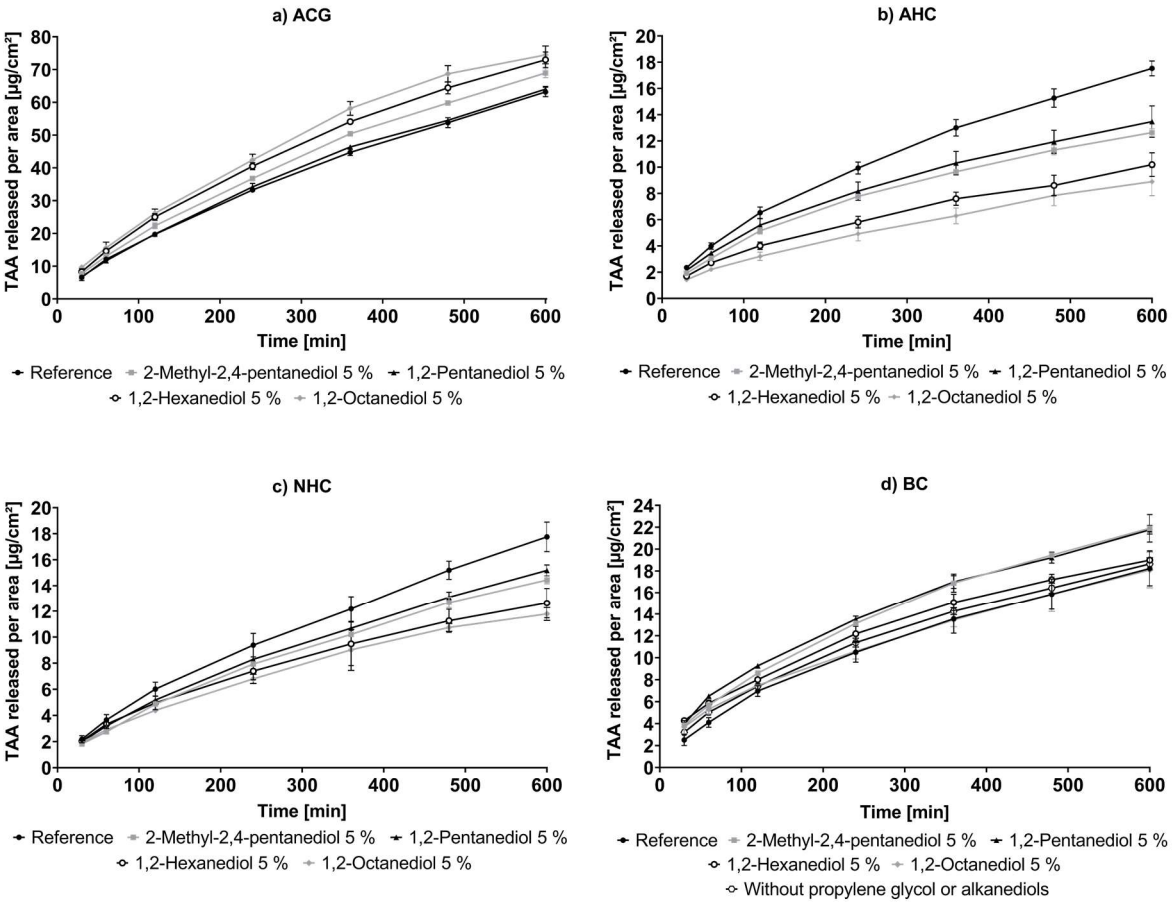


Fig. 4. TAA release per unit area [µg/cm^2] over time from (a) ACG, (b) AHC, (c) NHC, (d) BC, n = 3, mean ± standard deviation.

Fig. 4 shows the release profiles of TAA from the various formulations investigated. In all cases, the additon of alkandiols did not change the principal release characteristics. The TAA amounts released from the three cream bases at the end of the measurement period were in the same order of magnitude. By contrast, the carbomer gel (ACG) released fourfold higher amounts of TAA compared to the creams. For suspension-type formulations, the amount of drug released per unit area follows the Higuchi square root equation (Higuchi, 1961). As the micrographs proved that TAA is suspended in the bases, the experimental release data are presented accordingly (Fig. 5).

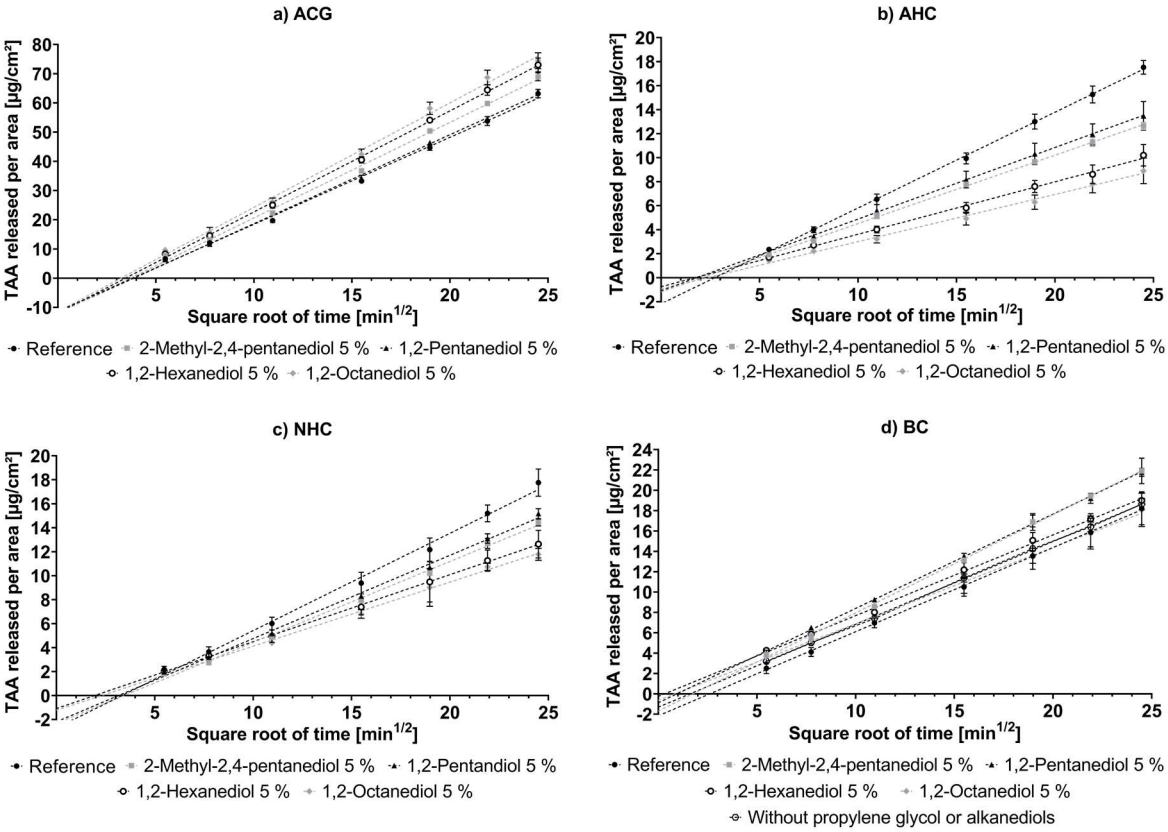


Fig. 5. TAA release per unit area [µg/cm^2] over the square root of time [min$^{1/2}$] from (a) ACG, (b) AHC, (c) NHC, (d) BC, n = 3, mean ± standard deviation.

Expectedly, the plots of the amount of TAA released per unit area [µg/cm^2] versus the square root of time [min^{½}] showed straight lines for all formulations. A linear regression analysis of these curves revealed coefficients of determination which are larger than 0.99 in all cases (see supplementary material) indicating that TAA release follows Higuchi kinetic.

Based on the data of the solubility of TAA in the aqueous phases, the dissolved TAA fraction in the outer phase of the different formulations can be calculated. If this dissolved fracion is compared to the amount of TAA released after 10 h it is found that the amount of TAA released after 10 h from all the formulations studied exceeds at least half of the initially available amount of TAA dissolved in the preparations used, it can be assumed that TAA is partially disolved from the suspended fraction during the experiment.

Consequently, the concentration of dissolved TAA remains constant within the preparation as long as suspended TAA is provided in excess. This conclusion can likewise be confirmed by the curves. Such behaviour is an important prerequisite for

the application of the Higuchi equation (Siepmann and Peppas, 2011). Accordingly, the corresponding release rates were calculated from the slopes of the linear regression of the Higuchi plot (Food and Drug Administration, 1997). The respective results are summarized in Table 3.

Table 3. Overview of the slopes of the curves for the TAA release over the square root of time.

Formulation	a) ACG	b) AHC	c) NHC	d) BC
Reference	2.978	0.7980	0.8117	0.8265
5% 2-Methyl-2,4-pentanediol	3.272	0.5694	0.6945	0.9650
5% 1,2-Pentanediol	3.045	0.5965	0.6755	0.9270
5% 1,2-Hexanediol	3.461	0.4371	0.5586	0.7936
5% 1,2-Octanediol	3.575	0.3933	0.5343	0.7506
Without propylene glycol or alkanediols	-	-	-	0.8141

Table 3 reveals that the TAA release from all ACG type formulations was significantly higher than from all creams irrespective of the addition of an alkandiol.

Equivalence of the release rates of a formulation and the respective reference formulation is postulated if the confidence interval of the median of all T/R ratios falls within the limits of 0.75 and 1.33. This range is represented by the dotted lines in Fig. 6 which depicts the median values of the T/R ratios as well as the confidence intervals obtained from data in Table 3.

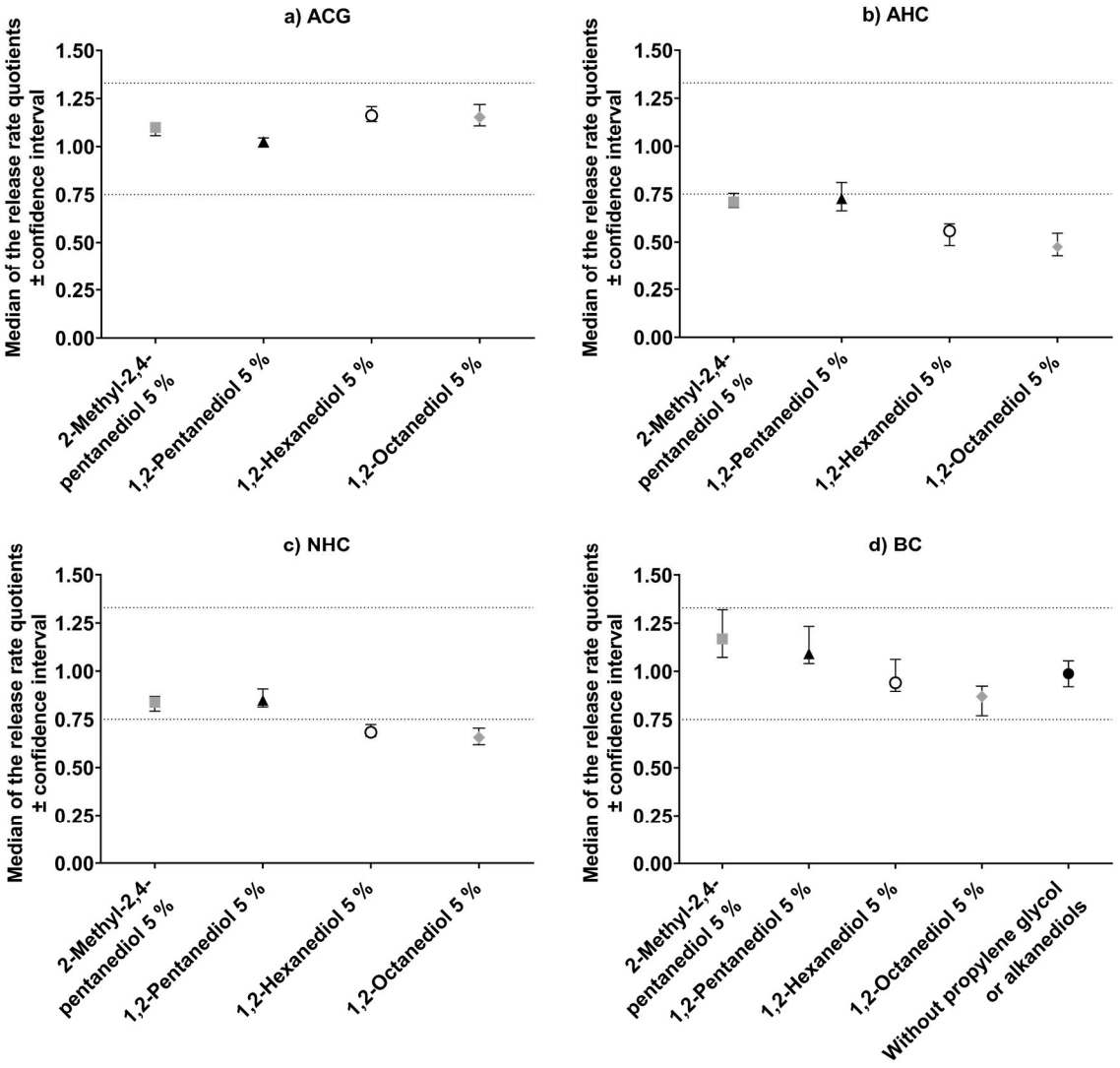


Fig. 6. Results of the equivalence test for comparison of the release rates of the tested formulations.

The release rates of the ACG variants comprising 5% of the tested alkanediols are equivalent to the reference formulation (Fig. 6a). All medians as well as all confidence intervals are within the respective limits. This result could have been expected because all ACG variants are single-phase basis containing TAA as a very finely dispersed solid. Due to this simple structure, no specific interaction between the API and the hydrogel occurs independent whether alkanediols were added or not. Moreover, the viscosity of the gels was not altered by the alkanediols (data not shown)[2]. Consequently, TAA is released at an equivalent rate from all hydrogels. Apart from that formal equivalence check, a closer look to the data reveals that TAA release rate was

[2] See Appendix, Chapter 8.1 Influence of Alkanediols on the Rheological Properties of the Aqueous Carbomer Gel

slightly increased upon the addition of the alkanediols. This can be attributed to the increased TAA solubility resulting in a marginally but statistically significant faster release compared to plain ACG. However, the significantly increased solubility of TAA with the addition of alkanediols does not affect the equivalence of release rates.

Results for the formulations based on the BC are shown in Fig. 6d. All medians as well as all confidence intervals are within the acceptance range represented by the dotted lines. Thus, despite the incorporation of the different alkanediols into BC, the release rates remain equivalent to the standard BC. However, it should be noted that the standard BC already contains 10% propylene glycol (1,2-propanediol). Therefore, a BC containing neither propylene glycol nor any other alkanediol was additionally tested to verify whether the effect studied is related to the presence of propylene glycol in the reference formulation. The release rate of TAA from BC containing neither propylene glycol nor any other alkanediol was comparable to that of the standard BC (containing 10% propylene glycol). Hence, surprisingly, propylene glycol seems to have only a marginal effect on the TAA release from BC. This result may explain why even longer-chained alkanediols have only a minor influence on the TAA release rate. The difference in concentration between 10% propylene glycol and 5% investigated alkanediols is therefore not relevant either. Apart from the formal check on equivalency, it is obvious that the release rate ratios continuously decrease with increasing chain length of the added alkanediol.

A more pronounced effect on the release rate was observed with the AHC based formulations (Fig. 6b). None of the formulation variants containing 5% of any of the studied alkanediols could prove equivalence to the reference formulation. Again, increasing chain length of the added alkanediol decreased the release rate.

This was also the case for the NHC formulation variants (Fig. 6c). However, the overall impact of the alkanediols was less pronounced. Therefore, the release rates of the formulations with the addition of 1,2-pentanediol and 2-methyl-2,4-pentanediol are still equivalent to the reference NHC without alkanediols. In contrast, the test on equivalency of the release rate could not be passed when the two longer-chained alkanediols, 1,2-hexanediol and 1,2-octanediol, were added to the NHC. These findings are in accordance with the results of a previous study showing that alkanediols are incorporated into the mixed crystal phase of the NHC (Sigg and Daniels, 2020).

The extent of the impact on the colloidal structure was found to be in the same order as the reduction of the TAA release with an augmenting effect with increasing chain length. The TAA solubility in the different NHC formulations was found to rise with increasing chain length of the added alkanediol (data not shown)[3]. Furthermore, the viscosity of the formulations is reduced with increasing chain length (Sigg and Daniels, 2020). Accordingly, it could be hypothesized that the TAA release should be increased as a function of the chain length of the added alkanediol.

However, TAA release from a hydrophilic cream starts from continuous aqueous phase. In contrast to the hydrogel where TAA crystals are found in a saturated aqueous solution, the drug in the creams is predominantly located in the inner, lipophilic phase. Therefore, partition and dissolution rate in the internal phase have a substantial impact on the release rate, as only the dissolved TAA fraction in the external, aqueous phase, is relevant for the TAA release from the cream. During the course of the release study, this amount depends not only on the equilibrium solubility of TAA in the aqueous phase but also on the subsequent delivery of released drug from the dispersed phase consisting of the lipid phase and mixed crystals. Obviously, the affinity of TAA for the dispersed phase increases with the interaction of the alkanediols with the dispersed phase, particularly with the mixed crystals. This comigration of TAA and alkanediol into the mixed crystals seems to be a major factor that influences TAA release. Accordingly, the release rate of TAA from these preparations is significantly reduced, even though the overall saturation solubility of TAA in NHC increases with the addition of alkanediols.

Since the colloidal structure of the AHC is very similar to that of the NHC, the incorporation of the alkanediols into the mixed crystal is also very likely to occur in case of AHC. Although all AHCs with alkanediols are outside the limits of 0.75 to 1.33, two different groups can be identified from Fig. 6b. The medians of the ratios of the release rates of the creams with 1,2-pentanediol and 2-methyl-2,4-pentanediol are both significantly higher than those of the formulations with 1,2-hexanediol and 1,2-octanediol. Similar as discussed with NHC, the ability of the alkanediols to interact with the mixed crystal directly affects TAA release. For all four alkanediols analysed,

[3] See Appendix, Chapter 8.2 TAA Solubility in Different Nonionic Hydrophilic Cream Formulations

the effect is more pronounced when with NHC resulting in release rates being not equivalent to the AHC without additives.

Summarizing the release analysis, the interaction of TAA with the liquid crystalline matrix of the cream formulations seems to increase with the addition of alkanediols, leading to a reduced TAA release. Even though that release from a formulation is not necessarily predictive of the partitioning into the stratum corneum and uptake into the living layers of the skin, where the site of action of TAA is located, drug release from dermal preparation is considered to be a major quality attribute (European Medicines Agency, 2018).

5.5. Conclusion

In the present study, the effect of four different alkanediols which can be used as alternative preservatives was investigated on the release of TAA as a model drug from three hydrophilic creams and one hydrogel. Although alkanediols increase the aqueous solubility of TAA, a substantial amount of the drug was found to be suspended in the semi-solid bases. Consequently, all formulations represent saturated solutions of the drug with suspended particles whereby the absolute quantities in the creams depend on the partition coefficient of TAA between aqueous phase, lipid phase and emulsifier rich liquid crystalline phases.

Due to the suspension-type character of the formulations, the amount of API released per unit area can be evaluated using the Higuchi equation (Higuchi, 1961) and was thus found to be proportional to the square root of time. Depending on the base, alkanediols modify the release of TAA. The addition of alkanediols to the ACG result in a slightly increased release rate with increasing alkyl chain length. In contrast, increasing chain length of the added alkanediols diminished the release rate from all tested creams. A formal test on equivalency revealed that the TAA release rates from ACG and BC variants were equivalent to the reference irrespective of the addition of an alkanediol to the formulation. The release rates of the NHC are equivalent to the standard formulation when adding the short-chained alkanediols, 1,2-pentanediol and 2-methyl-2,4-pentanediol, whereas they are different when adding longer-chained alkanediols. Finally, when AHC is used as a base all release rates were lowered to such an extent that none of the variants with alkanediols were equivalent to the reference.

Consequently, it is not possible to arbitrarily add alkanediols as alternative preservatives. Even though the addition of an alkanediol has only a minor influence on the stability and consistency of the formulation (Sigg and Daniels, 2020), the impact of the alkanediols on the aqueous solubility of the API and the interaction of the alkanediols with liquid crystalline phases in creams may impair the release to such an extent that the formulations cannot be considered to be equivalent. However, to see if this also translates into an altered drug penetration has to be clarified in future studies.

CRediT authorship contribution statement

Melanie Sigg: Conceptualization, Methodology, Investigation, Data curation, Writing - original draft, Visualization. Rolf Daniels: Conceptualization, Methodology, Resources, Supervision, Project administration, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Overview of the coefficients of determination R^2 of the curves for the release of TAA over the square root of time

Formulation	a) ACG	b) AHC	c) NHC	d) BC
Reference	0.9963	0.9994	0.9947	0.9996
5% 2-Methyl-2,4-pentanediol	0.9970	0.9966	0.9966	0.9992
5% 1,2-Pentanediol	0.9966	0.9992	0.9968	0.9992
5% 1,2-Hexanediol	0.9988	0.9973	0.9994	0.9982
5% 1,2-Octanediol	0.9959	0.9948	0.9971	0.9979
Without propylene glycol or alkanediols	-	-	-	0.9996

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6. Impact of Alkanediols on Stratum Corneum Lipids and Triamcinolone Acetonide Skin Penetration

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6.1. Abstract

Alkanediols are widely used as multifunctional ingredients in dermal formulations. In addition to their preservative effect, considering their possible impact on drug penetration is also essential for their use. In the present study, the influence of 2-methyl-2,4-pentanediol, 1,2-pentanediol, 1,2-hexanediol and 1,2-octanediol on the skin penetration of triamcinolone acetonide from four different semisolid formulations was investigated. Furthermore, confocal Raman spectroscopy measurements were performed to examine the influence of the alkanediols on stratum corneum lipid content and order. Alkanediols were found to increase the penetration of triamcinolone acetonide. However, the extent depends strongly on the formulation used. In certain formulations, 1,2-pentanediol showed the highest effect, while in others the penetration-enhancing effect increased with the alkyl chain length of the alkanediol used. None of the tested alkanediols extracted lipids from the stratum corneum nor reduced its thickness. Notwithstanding the above, the longer-chained alkanediols cause the lipids to be converted to a more disordered state, which favors drug penetration. This behavior could not be detected for the shorter-chained alkanediols. Therefore, their penetration-enhancing effect is supposed to be related to an interaction with the hydrophilic regions of the stratum corneum.

Keywords: alkanediols; preservation; triamcinolone acetonide; ex vivo skin penetration; stratum corneum lipids; confocal Raman spectroscopy (CRS)

6.2. Introduction

Many preservatives used in formulations for dermal application have fallen into disrepute, or have even been misreported because of undesirable effects, e.g., endocrine disrupting properties, during the last decade. Therefore, the demand for alternative preservatives is steadily increasing [1,2,3]. Alkanediols are promising candidates for this purpose. The most commonly used alkanediols are 1,2-pentanediol, 2-methyl-2,4-pentanediol (hexylene glycol), 1,2-hexanediol and 1,2-octanediol. Their preservative effect has already been demonstrated [4,5] and can primarily be attributed to their amphiphilic properties. With their hydrophilic head group on the one hand and their lipophilic alkyl chain on the other hand, they are enabled to interact and disorder the cell membranes of microorganisms [5]. Due to their amphiphilic nature, they can also interact with the surfactant and co-surfactant system of an emulsion or cream. This interaction becomes more intense with rising alkyl chain length [4,6]. In addition, the alkanediols used for alternative preservation must be safe and non-hazardous to health. The Cosmetic Ingredient Review Expert Panel rates the application of the alkanediols examined in our study as safe when used in the recommended concentration range [7,8]. Besides their preservative effect, they are known as multifunctional excipients exhibiting moisturizing and solubilizing properties [9,10].

Moreover, alkanediols are reported to act as penetration enhancers [11,12]. This is a particularly important aspect to be considered when using alkanediols in topical drug formulations. Therefore, the present study focused on this effect. The answer to this question is of paramount importance when an existing formulation is modified by substituting alkanediols for a conventional preservative. In accordance with the European Medicines Agency (EMA) Draft Guideline on quality and equivalence of topical products the impact on the dermal pharmacokinetic has to be assessed [13,14].

Triamcinolone acetonide (TAA), the model drug, is a glucocorticoid, that is widely used for treating inflammatory, allergic and pruritic dermatoses [15,16]. The glucocorticoid receptors which have to be addressed are located in the epidermal and dermal cells [17]. Therefore, after applying the formulation to the skin, TAA has firstly to overcome the stratum corneum (SC), which is the outermost permeability barrier of the skin, to reach its target structures. To this end, TAA is a perfectly suitable model drug for studying the influence of alkanediols on skin penetration.

Primarily, the penetration behavior of TAA as the active pharmaceutical ingredient (API) in different formulations and the effect of incorporating alkanediols was examined. Secondly, the impact of alkanediols on the lipid structures of the SC was investigated using confocal Raman spectroscopy (CRS).

As bases, we selected the same formulations as in the previous study focusing on the release of TAA [18], namely the nonionic hydrophilic cream (NHC), the anionic hydrophilic cream (AHC), and the aqueous carbomer gel (ACG) according to the German Pharmacopeia (DAB) [19] as well as the basic cream (BC) according to the German Drug Codex DAC (DAC) [20].

6.3. Materials and Methods

6.3.1. Materials

1,2-Pentanediol, 2-methyl-2,4-pentanediol, and 1,2-hexanediol (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany), polysorbate 60 (Kolliphor PS 60), cetostearyl alcohol (Kolliwax CSA), cetyl alcohol (Kolliwax CA), emulsifying cetostearyl alcohol type A (Kolliphor CSA) (BASF SE, Ludwigshafen, Germany), glycerol 85%, propylene glycol (Dr. Willmar Schwabe GmbH & Co. KG, Karlsruhe, Germany), white soft paraffin (Sasol Performance Chemicals, Hamburg, Germany), liquid paraffin (Hansen & Rosenthal KG, Hamburg, Germany), carbomer 50,000 (Fagron GmbH & Co. KG, Glinde, Germany), sodium hydroxide (Chemical supply of the University, Tuebingen, Germany), medium-chain triglycerides (Evonik Industries AG, Essen, Germany), glycerol monostearate 60, macrogol 20 glycerol monostearate, disodium hydrogen phosphate, potassium dihydrogen phosphate, sodium chloride, triamcinolone acetonide (micronized) (Caesar & Loretz GmbH, Hilden, Germany), bovine serum albumin (Carl Roth GmbH & Co. KG, Karlsruhe, Germany), acetonitril HPLC gradient grade (Honeywell Specialty Chemicals Seelze GmbH, Seelze, Germany), PTFE filters (pore size: 0.2 µm, diameter: 25 mm, Chromafil Xtra H-PTFE-20/25, Macherey-Nagel GmbH & Co. KG, Dueren, Germany), Neg-50 Frozen Section Medium (Thermo Fisher Scientific Inc., Waltham, MA, USA). 1,2-Octanediol (dermosoft Octiol) was kindly donated by Evonik Dr. Straetmans GmbH, Hamburg, Germany.

6.3.2. Preparation of Test Formulations

Table 1 provides an overview over the compositions of the compendial formulations examined in the present study. From these bases, the test formulations were prepared by adding 0.1% TAA, representing the upper recommended usage concentration [16]. In addition, all preparations were produced with an addition of 5% of the respective alkanediol. The amount of alkanediol added was deducted from the water amount. The reference BC contains 10% propylene glycol, which is likewise an alkanediol. Thus, 10% propylene glycol were substituted with 5% of the examined alkanediols to isolate the effect of the studied alkanediols from that of propylene glycol. The remaining 5% were replaced by water. The detailed manufacturing processes of each formulation are described in Sigg and Daniels [18].

Table 1. Composition (in % *w*/*w*) of the compendial bases without TAA and alkanediols.

(a) ACG		(b) AHC		(c) NHC		(d) BC	
Carbomer 50.000	0.5	Emulsifying cetostearyl alcohol type A	9.0	Polysorbate 60	5.0	Glycerol mono-stearate 60	4.0
Sodium hydroxide solution 5%	3.0	Liquid paraffin	10.5	Cetostearyl alcohol	10.0	Cetyl alcohol	6.0
Purified water	96.5	White soft paraffin	10.5	White soft paraffin	25.0	Medium-chain triglycerides	7.5
		Purified water	70.0	Glycerol 85%	10.0	White soft paraffin	25.5
				Purified water	50.0	Macrogol 20 glycerol monostearate	7.0
						Propylene glycol	10.0
						Purified water	40.0

ACG: aqueous carbomer gel, AHC: anionic hydrophilic cream, NHC: nonionic hydrophilic cream, BC: basic cream.

6.3.3. Dermatomed Pig Ear Skin

Porcine ear skin was used for the penetration experiments because it is similar to the human skin in terms of histology and morphology [21,22,23]. The ears used for the experiments were provided by either a local butcher (Bio Metzgerei Griesshaber, Moessingen-Oeschingen, Germany) or by the University Hospital Tuebingen (Department of Experimental Medicine, Tuebingen, Germany). The Institute of Pharmaceutical Technology is registered for the use of animal products with the Tuebingen District Office (registration number: DE 08 4161052 21, approval date: 22 December 2015). Prior to and after cutting the full-thickness skin from the cartilage, it was cleaned with isotonic saline and cotton swabs. The skin was cut into strips approximately 4 cm wide and fixed with pins on a Styrofoam plate wrapped with

aluminum foil. The hair was trimmed to approximately 0.5 mm using a hair clipper (QC5115/15, Philips, Amsterdam, The Netherlands). After cutting the skin to a thickness of 1 mm with a dermatome (GA 630, Aesculap AG & Co. KG, Tuttlingen, Germany), circles with a diameter of 25 mm were punched out from the prepared strips. Subsequently, the skin punches were wrapped in aluminum foil and stored at −30 °C until the day of the experiment.

6.3.4. Ex Vivo Penetration Studies

Ex vivo penetration studies were performed with modified Franz diffusion cells (Gauer Glas, Puettlingen, Germany), having a receptor volume of 12 mL as well as an inner diameter of 15 mm, representing an actual penetration area of 1.77 cm².

Owing to its high lipophilicity, the solubility of TAA in phosphate-buffered saline (PBS) is not high enough to achieve sink conditions in the experiments presented below [18]. Therefore, to increase the TAA solubility, 4% (w/w) bovine serum albumin (BSA) was added to PBS. The acceptor medium was warmed to 32 °C, representing the skin surface temperature, and stirred at a speed of 500 rpm. The Franz cells were equipped with dermatomed porcine skin. Approximately 15 mg of the formulation were applied to the skin after an equilibrium period of 30 min to work under finite dosing conditions [24,25]. The formulation was evenly distributed on the skin by means of a plexiglas cylinder and the mass of the applied preparation was determined accurately.

An incubation period of 24 h was chosen, as TAA is usually applied only once per day because of its depot effect [26,27]. Thereafter, the skin samples were detached from the Franz diffusion cells. The formulation remaining on the skin surface was wiped away with a cotton swab soaked with 500 µL of isotonic saline. After the penetration area (diameter: 15 mm) has been punched out, the skin pieces were weighted and immediately frozen in aluminum molds in liquid nitrogen to prevent further penetration.

The dissection of the skin was carried out by means of a cryo-microtome (HM 560 Cryo-Star; Thermo Fisher Scientific Inc., Waltham, MA, USA). After the frozen skin samples were fixed to the sample holder using a frozen section medium (NEG 50; Thermo Fisher Scientific Inc., Waltham, MA, USA), the skin was cut into slices having a thickness of 16 µm. The first incomplete as well as the first complete section are assigned to the SC. The following 14 sections represent the living epidermis. The last

sections belong to the dermis [28,29,30]. The segments were collected and extracted with 1 mL acetonitrile. After vortexing (Vortex 2, IKA-Werke GmbH & Co. KG, Staufen, Germany) for 10 s, the samples were further extracted in an ultrasonic bath for 30 min. The extraction medium was then withdrawn and filtered through a 0.2 µm PTFE filter (Chromafil Xtra H-PTFE-20/25, Macherey-Nagel, Dueren, Germany). The TAA content was analyzed by HPLC (high-performance liquid chromatography) as described below.

The cotton swab used for wiping the remaining formulation was extracted with 9.5 mL of acetonitrile by vortexing for 1 min, sonication for 30 min and subsequently vortexing again for 1 min. The HPLC analysis was performed after filtration through a 0.2 µm PTFE filter (Chromafil Xtra H-PTFE-20/25, Macherey-Nagel, Dueren, Germany).

Formulation residues that stick to the top of the Franz cells were removed with a second cotton swab. This swab was extracted with the same extraction method used for the first cotton swab. The determined API amount detected on the second cotton swab was then subtracted from the initially applied amount yielding the final amount of API applied to the skin.

Samples were also taken from the acceptor medium to determine permeated TAA. Acetonitrile was added to the sample (3 + 1) to remove BSA prior to HPLC analysis [31]. Following 10 s of vortexing (Vortex 2, IKA-Werke GmbH & Co. KG, Staufen, Germany), samples were centrifuged (MiniSpin, Eppendorf AG, Hamburg, Germany) at 13,400 rpm for 15 min to pellet BSA. Subsequently, an HPLC analysis of the clear supernatant was performed.

Experiments were performed in triplicate.

6.3.5. HPLC Analysis

The different TAA solutions were analyzed using an HPLC system (LC 20AT, DGU 20A5R, SIL 20AC HAT, CTO 10ASVP, CBM 20 A, Shimadzu GmbH, Duisburg, Germany) in combination with an UV detector (SPD 20A, Shimadzu GmbH, Duisburg, Germany). For TAA quantification, the separation was conducted at 30 °C on a RP-18 column (Nucleosil 100-5 C18 125/4, Macherey-Nagel GmbH & Co. KG, Dueren, Germany). The mobile phase was acetonitrile-water (40:60, v:v). The flow rate was adjusted to 0.8 mL/min, and 20 µL sample volume was injected. UV absorption was determined at a wavelength of 254 nm. The TAA retention time was 4.8 min.

The limit of quantification (LOQ) was calculated according to the ICH (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use) guidelines [32]. The LOQ for the permeation samples was 0.2406 µg/mL and for the penetration samples 0.0254 µg/mL, respectively.

6.3.6. Calculation of the Penetrated TAA Amount

The calculation of the penetrated TAA amount is normalized to an applied amount of 15 mg formulation as well as to a mass of the skin piece of 175 mg. Further, the penetrated TAA mass is averaged across the overall punched out skin piece. Although not explicitly indicated all penetration experiments are referred to the same time interval.

The quantity of TAA penetrating into the skin piece per unit area was calculated according to Equation (1).

$$m(\text{TAA})\left[\frac{\text{ng}}{\text{cm}^2}\right] = \sum \frac{c(\text{TAA}) \cdot V(\text{acetonitrile}) \cdot 15\ \text{mg} \cdot 175\ \text{mg}}{m(\text{formulation}) \cdot m(\text{total skin piece}) \cdot 1.77\ \text{cm}^2} \quad (1)$$

where in c(TAA) is the concentration of TAA determined by HPLC, V(acetonitrile) is the volume of acetonitrile used to extract the skin segments, m(formulation) indicates the exact mass of preparation applied and m(total skin piece) denotes the accurate mass of the respective total skin piece. The calculation was performed separately for SC, epidermis and dermis and the cumulative amount of penetrated TAA was assessed by summing up data from all three layers.

Accordingly, the penetrated fraction of TAA relative to the totally applied TAA mass was calculated according to Equation (2).

$$m(\text{TAA})[\%\ \text{of applied amount}] = \frac{c(\text{TAA}) \cdot V(\text{acetonitrile}) \cdot 15\ \text{mg} \cdot 175\ \text{mg}}{m(\text{formulation}) \cdot m(\text{total skin piece}) \cdot m(\text{TAA, applied})} \quad (2)$$

6.3.7. Incubation of the Skin Samples with Alkanediol Solutions

The same setup as for the ex vivo penetration studies was used for the incubation of the skin samples with the alkanediol solutions. Three skin pieces were incubated with 1 mL of 5% solutions of 2-methyl-2,4-pentanediol, 1,2-pentanediol and 1,2-hexanediol, respectively. Owing to its reduced solubility in water, 1,2-octanediol was applied as a saturated solution. After an incubation period of 24 h, the skin samples were removed from the Franz diffusion cells. The remaining solutions were washed off the skin using

cotton swabs soaked with PBS for 30 times. Afterwards, the incubation area was punched out and dabbed dry with cotton swabs.

6.3.8. Preparation of Isolated Stratum Corneum (SC)

The SC was isolated according to a method described by Kligman and Christophers [33] and Zhang and Lunter [34], which does not to affect the lamellar lipid organization of the SC lipids [35]. The skin was placed dermal side down on a filter paper soaked with 0.2% trypsin solution. After 24 h, the digested material was removed with two blunt forceps. The isolated SC was immersed in a 0.05% trypsin inhibitor solution for one minute before being washed five times in succession with highly purified water. The SC sheets obtained were picked up on glass slides and dried over silica gel in a desiccator for at least 72 h.

6.3.9. Confocal Raman Spectroscopy (CRS) Measurements

Measurements with a confocal Raman spectrometer (CRS) were performed using a method described by Liu and Lunter [36,37,38] and Zhang and Lunter [34] to analyze the impact of alkanediols on the SC lipids and the SC thickness. The SC sheets, dried for at least 72 h, were applied to the scanning table of the Raman microscope (alpha 500 R confocal Raman microscope, WITec GmbH, Ulm, Germany). The CRS device was equipped with a 532 nm excitation laser, UHTS (ultra-high throughput) 300 spectrometer and a DV401-BV CCD (charge coupled device) camera. The laser power was set to 10.00 mW with an optical power meter (PM100D, Thorlabs GmbH, Dachau, Germany) before starting the measurements to avoid skin damage due to burning. A 100× objective with a numerical aperture of 0.9 (EC Epiplan-neofluor, Carl Zeiss, Jena, Germany) was used for focusing the excitation radiation onto the skin samples. The backscattered light from the skin samples was spectrally analyzed by an optical grating (600 g/mm) of the spectrometer and was then projected onto the detector of the CRS. The detector was cooled down to −60 °C in advance. The spectra were collected with an integration time of 4 s and 2 accumulations.

Three SC sheets were measured for each alkanediol solution, and spectra were recorded at three random locations for each SC sheet. This resulted in a total of nine measurements for each solution.

6.3.9.1. Determination of the Skin Thickness

For determination of the SC thickness, the keratin signal (v(CH_3), 2920–2960 cm^{-1}) was used. The focus point was moved from 15 µm under a SC sheet to 15 µm above with a step size of 1 µm. The area under the curve (AUC) of the keratin peak was calculated and plotted against the depth. The full width at half maximum (FWHM) of this peak served as skin sample thickness [36,37].

When using a dry objective, it was observed that the determined SC thicknesses are generally too low compared to the real thicknesses [37]. Native SC contains water. This means that the corneocytes are filled with water and therefore swollen, and thus more voluminous than in the dried SC. Hence, a higher thickness of native SC compared to dried SC is a consequence of the different water content [39]. However, it was also shown that the used CRS configurations result only in an overall decrease in the measured thickness values [37]. When referring the test samples to a reference sample, the thickness variations caused can be eliminated. Consequently, when comparing test samples to a reference, the conclusions are still correct.

6.3.9.2. Analysis of Lipid Content

Figure 1 represents a typical CRS spectrum of an SC sheet.

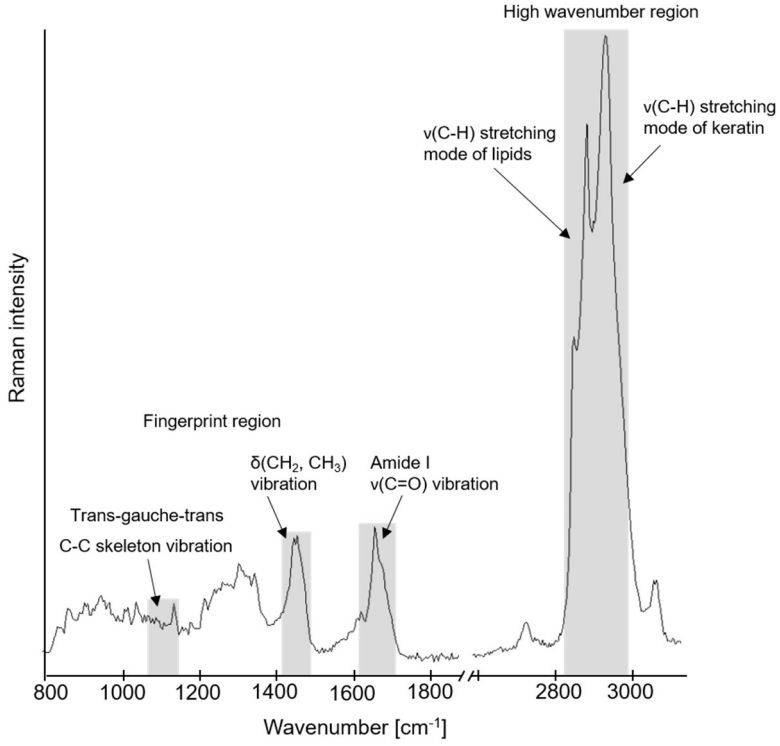

Figure 1. Typical confocal Raman spectroscopy spectrum of stratum corneum with highlighted peaks used for calculation of lipid content and order.

In the fingerprint region of the Raman spectrum of the SC samples, the peak from 1425 to 1490 cm^{-1} belongs to δ(CH_2, CH_3)-vibration mode and the one ranging from 1630 to 1710 cm^{-1} to ν(C=O)-mode for Amide I. The latter shows the lowest variation within different measurements of one donor pig as well as between various SC samples originating from different donor pigs. For this reason, this peak is used for normalization [34].

The δ(CH_2, CH_3) signal 1425–1490 cm^{-1} arises from lipids and proteins and is considered as lipid peak [34,40]. It is used for calculating the lipid amount, related to the Amide I peak according to Equation (3).

$$\text{Normalized signal (fingerprint region)} = \frac{AUC_{1425\text{-}1490}}{AUC_{1630\text{-}1710}} \quad (3)$$

In addition, the peaks in the high wavenumber region were examined to obtain further information about the lipid content. In this region the signals originating from keratin and those caused by lipids overlap. Therefore, a Gaussian deconvolution is necessary to separate the content of these peaks. The deconvolution was performed as described in Liu and Lunter [36]. The peaks between 2930 cm^{-1} and 2980 cm^{-1} arise from keratin, whereas the peaks in the range of 2850 cm^{-1} to 2880 cm^{-1} derive from the ν(C-H) symmetric and ν(C-H) asymmetric stretching modes of the lipids, respectively. As in the fingerprint region, these keratin peaks are also employed for normalizing the lipid peaks and determining the lipid content [36,41]. The normalized lipid signal is calculated according to Equation (4).

$$\text{Normalized signal (high wavenumber region)} = \frac{AUC_{2850}+AUC_{2880}}{AUC_{2930}+AUC_{2980}} \quad (4)$$

The calculated results of the normalized signals can be used to identify variations of the SC lipid content.

6.3.9.3. Analysis of Lipid Order

In the fingerprint region, three peaks are associated with the C-C skeleton vibration mode of the long-chained hydrocarbons. These modes are located at wavenumbers of 1060 cm^{-1}, 1080 cm^{-1} and 1130 cm^{-1}. The peaks at 1060 cm^{-1} and 1130 cm^{-1} correspond to the all-trans conformation which represents the more ordered state of the lipids. The peak at 1080 cm^{-1} corresponds to the gauche conformation and represents the more disordered state of the lipids [42,43,44]. When the lipids are

converted from the ordered to the disordered state, the signal of the peak at 1060 cm^{-1} becomes weaker, while the shape of the peak at 1080 cm^{-1} gets broader [42].

For reducing the noise and for augmenting the signal-to-noise ratio of the obtained result, a principal component analysis and a polynomial background subtraction are necessary [36]. Moreover, the peak at 1130 cm^{-1} contains part of the weak keratin peak at 1125 cm^{-1} [36,42]. As a result, an adequate integration area has to be selected in order to eliminate the influence of this keratin contribution [36].

The conformational order was calculated using Equation (5) [36,42,44].

$$\text{Conformational order (fingerprint region)} = \frac{AUC_{1080}}{AUC_{1060}+AUC_{1130}} \quad (5)$$

Accordingly, a high value of the conformational order represents a predominant gauche conformational order (less-ordered lateral packing of lipids), while a low value is indicative of a trans conformational order (higher-ordered lateral packing of lipids).

6.3.10. Statistical Analysis

The following graphs represent the arithmetic mean ± standard deviation (mean ± SD) extracted from triplicate measurements of the ex vivo skin penetration experiments, or the spectral data obtained from nine independent measurements, respectively. Statistical differences in the total TAA amount in all skin layers as well as differences in the data obtained from CRS measurements were analyzed with a one-way ANOVA (analysis of variance) followed by Tukey's multiple comparisons test. To consider significant differences in the amount of TAA penetrated into each skin layer, a two-way ANOVA followed by Tukey's multiple comparisons test was performed. The significant differences are presented with a different number of asterisks (*) as follows: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$. Only the differences related to the reference formulations are marked with asterisks in the graphs for better clarity. The statistical significances between the formulations with alkanediols are displayed in the supplementary material.

6.4. Results

6.4.1. Skin Permeation

In all penetration experiments performed the acceptor fluid has been analyzed for permeated TAA. In all cases the concentration of TAA in the acceptor medium was below the LOQ of the HPLC method. This has been expected, since TAA is a highly lipophilic drug (logP 2.53 [45]) and its permeation through the skin is reduced compared to hydrophilic APIs [46,47,48]. Moreover, it is known from previous studies that TAA accumulates in the SC forming a reservoir [49].

6.4.2. Skin Penetration

Figure 2 represents the results of the TAA penetration experiments from the various ACG formulation variants. The data reveals that the addition of alkanediols significantly increases TAA penetration. Moreover, there is a clear trend that the penetration rates increase with increasing alkyl chain length of the added alkanediol, with 1,2-octanediol showing the most pronounced penetration enhancement. Further, in these samples a significantly increased TAA penetration is also detected in the dermis, whereas the other alkanediols only significantly increased the TAA amount in SC and epidermis. Despite the differences in detail, it is evident from Figure 2 that all alkanediols studied lead to an increased TAA penetration.

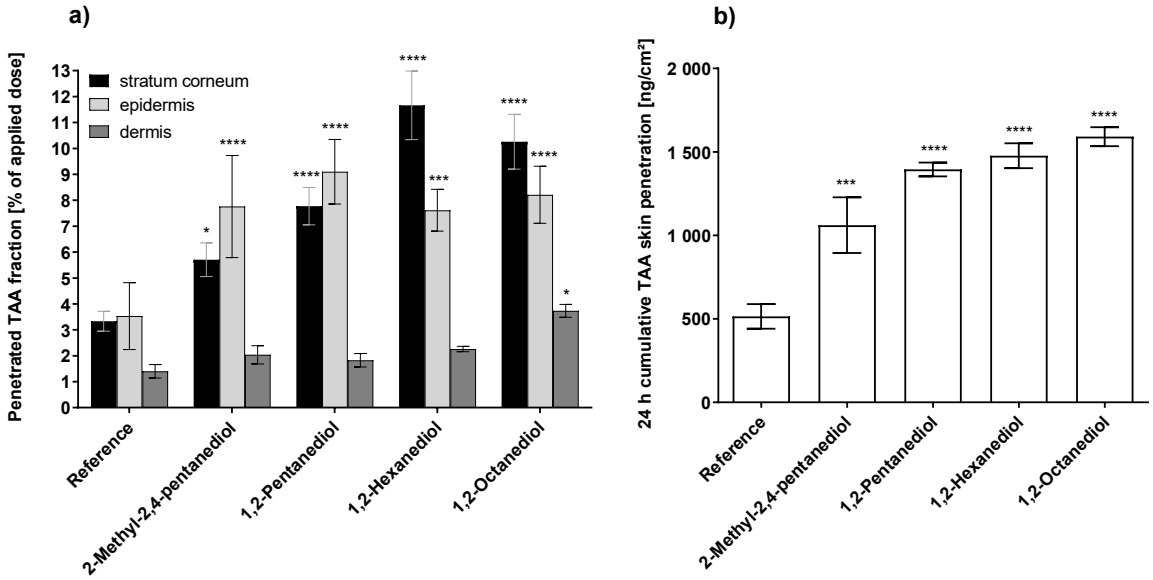

Figure 2. Triamcinolone acetonide penetration from pure aqueous carbomer gel (reference) and aqueous carbomer gel with 5% alkanediol after 24 h incubation; (a) Penetrated fraction in stratum corneum, viable epidermis and dermis; (b) Cumulative skin penetration of triamcinolone acetonide, n = 3, mean ± SD, * $p \leq 0.05$; *** $p \leq 0.001$; **** $p \leq 0.0001$.

The impact of alkanediols on TAA penetration from AHC is summarized in Figure 3. As can be seen in Figure 3a, 2-methyl-2,4-pentanediol does not significantly vary the TAA penetration within the different skin layers. However, the impact on the total skin penetration is significantly higher compared to the referenced formulation as a consequence of the different calculation methods applied in Figure 3a,b. 1,2-Pentanediol shows the highest penetration rates when considering the three skin layers individually as well as when considering penetration in the whole skin. Both, 1,2-hexanediol and 1,2-octanediol increase the TAA penetration from AHC to a similar extend. The numerical values are intermediate between the ones of 2-methyl-2,4-pentanediol and that of 1,2-pentanediol.

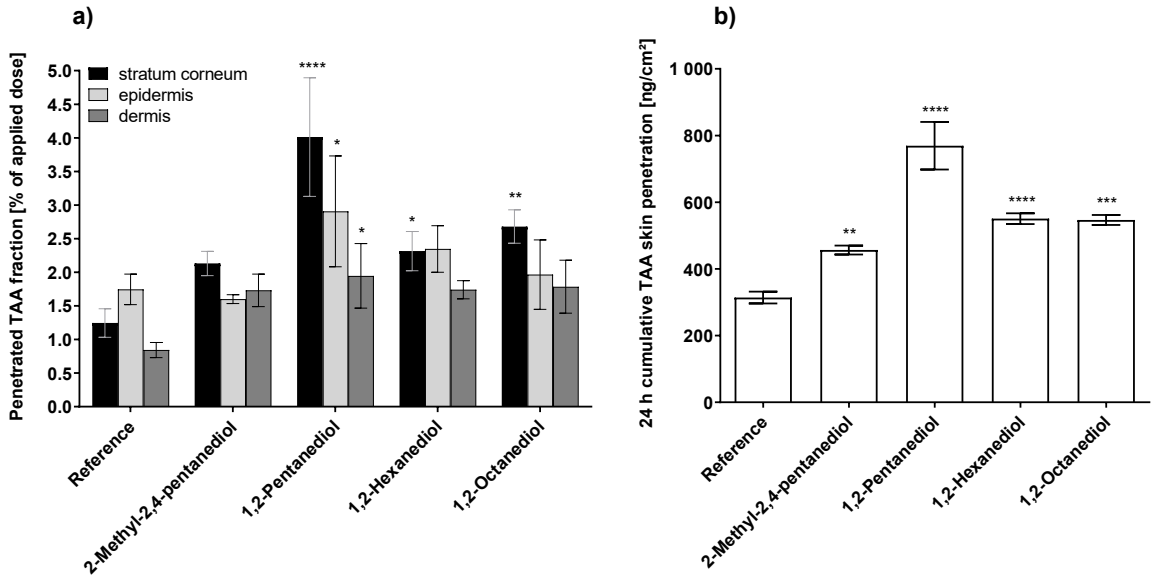

Figure 3. Triamcinolone acetonide penetration from pure anionic hydrophilic cream (reference) and anionic hydrophilic cream with 5% alkanediol after 24 h incubation; (a) Penetrated fraction in stratum corneum, viable epidermis and dermis; (b) Cumulative skin penetration of triamcinolone acetonide, n = 3, mean ± SD, * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$.

Figure 4 depicts the effect of alkanediol addition on TAA penetration from NHC. It can be clearly seen that the longer-chained alkanediols, 1,2-hexanediol and 1,2-octanediol, significantly increase both the TAA fraction penetrated in the different skin layers as well as the cumulative skin penetration expressed as absolute amount of TAA present in the skin after 24 h. The addition of 2-methyl-2,4-pentanediol does not result in a significantly larger penetration. However, this finding seems to be mostly due to the larger standard deviation. Further, 1,2-pentanediol shows an increased cumulative TAA skin penetration. However, the effect is significantly less pronounced compared to the longer-chained alkanediols. Additionally, in contrast to Figure 2a and Figure 3a,

the TAA distribution of the reference is reproduced in the NHC containing the investigated alkanediols. The TAA concentration is highest in the viable epidermis in all samples investigated.

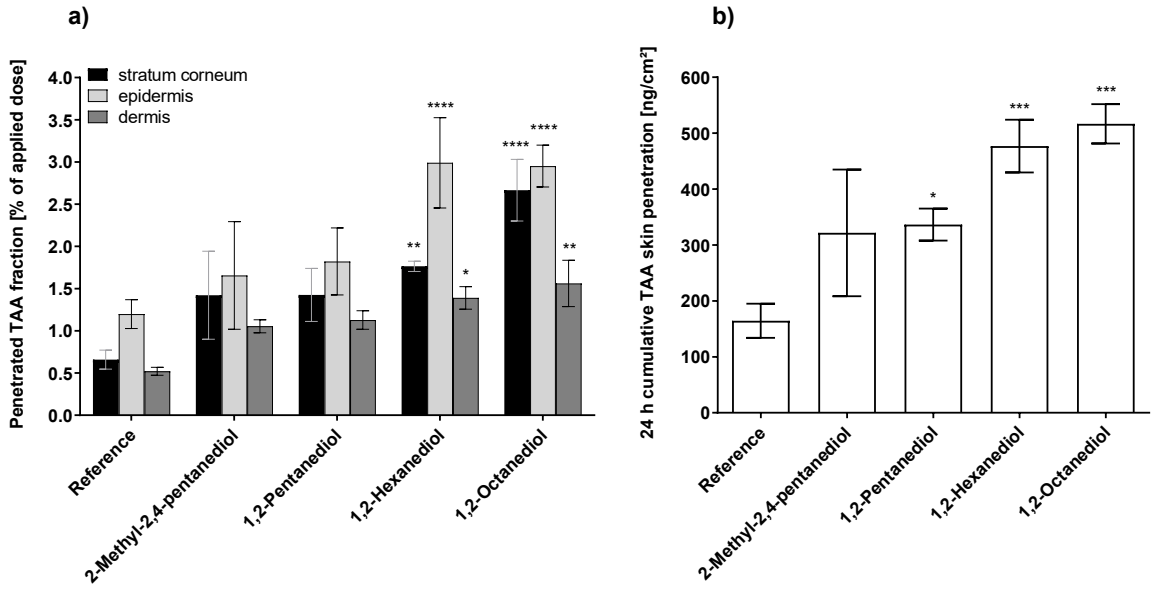

Figure 4. Triamcinolone acetonide penetration from pure nonionic hydrophilic cream (reference) and nonionic hydrophilic cream with 5% alkanediol after 24 h incubation; (a) Penetrated fraction in stratum corneum, viable epidermis and dermis; (b) Cumulative skin penetration of triamcinolone acetonide, n = 3, mean ± SD, * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$.

As can be taken from Figure 5, generally, the effect of alkanediols on the TAA penetration from BC is small. 1,2-Pentanediol is the only alkanediol that significantly increases the penetration of TAA. All other alkanediols do not enhance the TAA penetration. It is worth mentioning, that the compendial BC reference formulation contains 10% propylene glycol, which was replaced by 5% of the respective alkanediols to obtain the test formulations. The remaining 5% were supplemented with water. As propylene glycol itself is also known to act as penetration enhancer [50], the impact of the added alkanediols on TAA penetration is expectedly low. Only 1,2-pentanediol shows a small but significant enhanced penetration compared to the reference formulation containing propylene glycol.

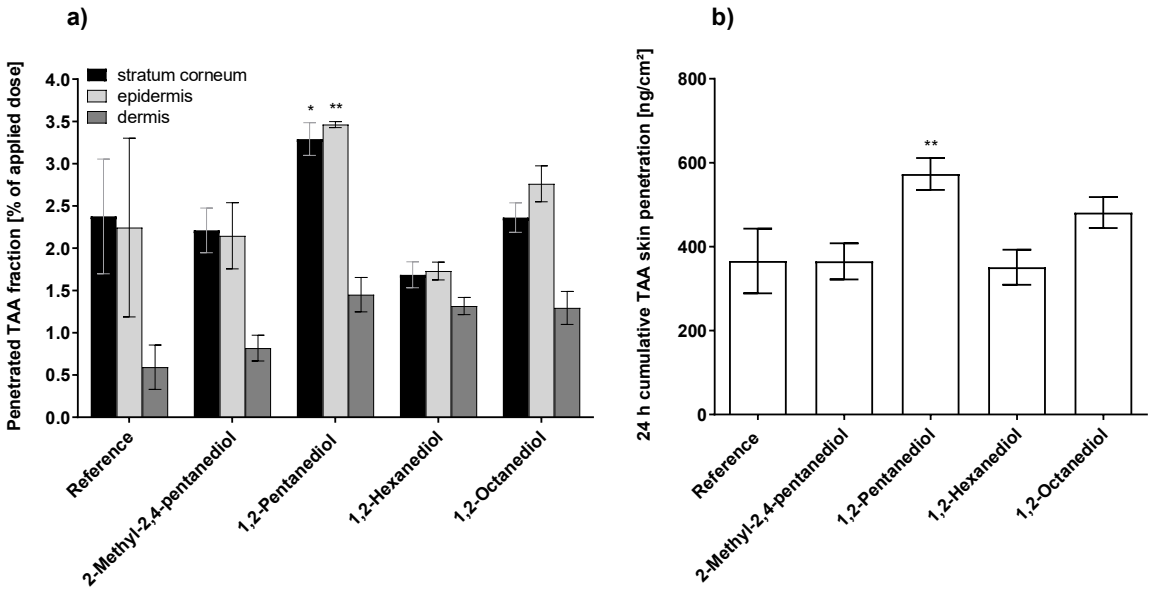

Figure 5. Triamcinolone acetonide penetration from pure basic cream (reference, containing 10% propylene glycol) and basic cream with 5% alkanediol after 24 h incubation; (a) Penetrated fraction in stratum corneum, viable epidermis and dermis; (b) Cumulative skin penetration of triamcinolone acetonide, n = 3, mean ± SD, * $p \leq 0.05$; ** $p \leq 0.01$.

6.4.3. Confoal Raman Spectroscopy Measurements

6.4.3.1. Lipid Content and Stratum Corneum Thickness

The amphiphilic nature of alkanediols enables them to interact with the microbial cell membranes [51] as well as with the surfactant and co-surfactant system of the cream bases used [4]. Thus, it appeared very likely that they also interact with SC lipids. To investigate this interaction, CRS measurements were conducted.

Figure 6 depicts the normalized lipid signals which represent the lipid content of the SC after 24 h incubation with the respective alkanediol solutions in comparison to pure water.

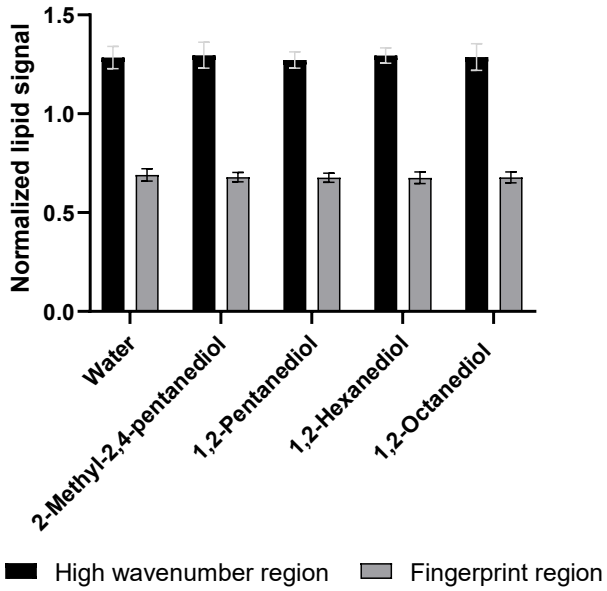

Figure 6. Normalized lipid signals in high wavenumber and fingerprint regions for lipid content analysis, after treatment with water and 5% solutions of 2-methyl-2,4-pentanediol, 1,2-pentanediol and 1,2-hexanediol, and saturated 1,2-octanediol solution, n = 9, mean ± SD.

From these results it is obvious, that the treatment with alkanediols does not alter the SC lipid content in comparison to incubation with water. The results for the lipid content analysis in the high wavenumber and fingerprint regions are identical. This indicates that the tested alkanediols are not able to extract lipids from the SC.

As already described above, the thicknesses measured using this CRS methodology do not correspond to the “real” thicknesses of the SC. The measured values are all systematically lower than the values measured on SC sheets not prepared as necessary for this method. However, despite this systematic deviation, conclusions are valid as far as the results are seen in relation to a respective reference sample, i.e., water treated specimen. It can be clearly taken from Figure 7 that all measured thicknesses after alkanediol treatment do not differ significantly from the water treated samples. Thus, it can be concluded that the alkanediols do not have an impact on the SC thickness. These findings are in good agreement with the results for the lipid content analysis. As thinning of the SC is usually linked to lipid extraction [34] and as no lipids were extracted by the alkanediols, the SC thicknesses do not shrink either.

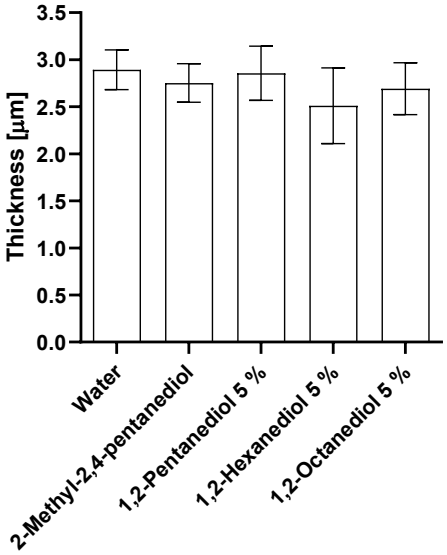

Figure 7. Measured thicknesses of SC after treatment with water and 5% solutions of 2-methyl-2,4-pentanediol, 1,2-pentanediol and 1,2-hexanediol, and saturated 1,2-octanediol solution, n = 9, mean ± SD.

6.4.3.2. Lipid Order

Figure 8 presents the alteration of SC lipid order caused by the alkanediol solutions. The peak at 1080 cm^{-1} represents the gauche conformation whereas the peaks at 1060 cm^{-1} and 1130 cm^{-1} correspond to the all-trans conformation of the lipids. A rising ratio of these signals indicates an increase in the gauche fraction representing a more disordered state of the SC lipids.

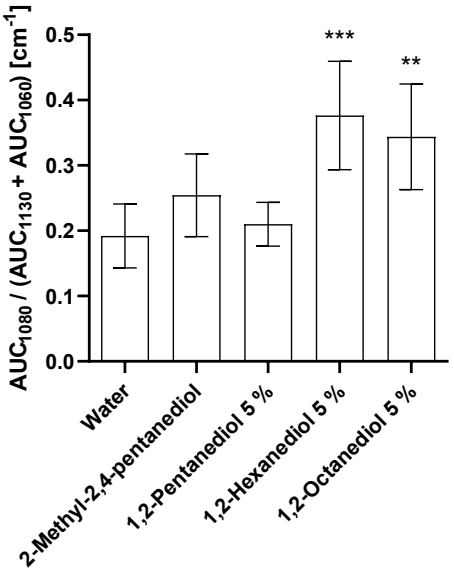

Figure 8. C-C skeleton vibration mode: calculation of ratio for lipid order analysis, after treatment with water and 5% solutions of 2-methyl-2,4-pentanediol, 1,2-pentanediol and 1,2-hexanediol, and saturated 1,2-octanediol solution, n = 9, mean ± SD, ** $p \leq 0.01$; *** $p \leq 0.001$.

1,2-Pentanediol and 2-methyl-2,4-pentanediol do not show a significant effect on the lipid order compared to the water reference. In contrast, the longer-chained alkanediols, 1,2-hexanediol and 1,2-octanediol lead to a significant rise in this ratio, indicating a less-ordered state of the lipids. Thus, the alteration of lipid order depends on the alkyl chain length of the alkanediol applied. The shorter-chained alkanediols do not seem to be able to intercalate into the SC lipids, whereas the alkyl chain of the longer-chained ones seems to be long enough for an intercalation. As a result, the SC lipids become disordered.

6.5. Discussion

The target location of topically applied glucocorticoids, which is the glucocorticoid receptor, is located in the living skin layers [17]. Therefore, when applying the TAA-containing formulations, TAA has to overcome the SC, forming the main entrance barrier, before being able to reach its site of action in the deeper skin layers.

Release from the semisolid preparation is the first step for the transport of an API into the skin. Detailed TAA release experiments from the same creams used in the present study revealed a general decrease when adding alkanediols. In contrast, TAA release increased when alkanediols were added to the ACG hydrogel [18]. This underlines as already known from diverse research [52,53,54] that API release from a dermal formulation is an important quality factor but does only partially describe the biopharmaceutical properties of a dermal preparation.

The presented results reveal that alkanediols affect TAA penetration in various ways depending on the base formulation considered.

In general, the penetration of a drug can be enhanced by increasing its thermodynamic activity in the vehicle. As known from previous studies, 0.1% TAA is above its saturation in all formulations studied yielding a suspension of undissolved TAA in a saturated vehicle [18]. Accordingly, the thermodynamic activity of TAA in all tested preparation equals 1. Consequently, a larger or smaller fraction of undissolved TAA does not alter its thermodynamic activity and thus cannot be the cause for the observed penetration enhancement [55,56].

Thus, it seems to be more likely that the increased penetration of TAA in the presence of alkanediols is due to an increased solubility of the drug in the SC. Alkanediol molecules are known to penetrate the SC and increase the overall API solubility in the skin barrier [57,58]. This would shift the TAA distribution towards the SC. Therefore, the respective solubility parameters of TAA, the alkanediols and the SC are considered and summarized in Table 2. The solubility parameters δ allow to make a theoretical prediction of the solubility of compounds in one another. In the present study, these parameters predict the solubility of TAA in the alkanediols and in the SC. The lower the difference in δ-values between TAA and a substance, the higher the solubility of TAA in the corresponding substance [59]. Correspondingly, the solubility of TAA increases when Δδ approximates zero.

Table 2. Solubility parameters (δ) of the substances and differences of solubility parameters between the alkanediols, stratum corneum and triamcinolone acetonide (Δδ).

	δ [(MPa)^{½}]	**Δδ [(MPa)^{½}]**
Triamcinolone acetonide	20.10 [a]	-
2-Methyl-2,4-pentanediol	26.79 [b]	6.66
1,2-Pentanediol	24.98 [b]	4.88
1,2-Hexanediol	24.13 [b]	4.03
1,2-Octanediol	22.92 [b]	2.82
Stratum corneum	21.81 [c,d]	1.71

[a] Abou-ElNour et al. [60]; [b] Sigg and Daniels [18]; [c] Ezati et al. [61], [d] Venkatram et al. [62].

As can be taken from Table 2, the difference in the solubility parameters between TAA and the SC is very low (1.71 (MPa)^{½}), indicating a high affinity of TAA for this skin layer [63]. This is associated with TAA accumulation and consequently a relatively high fraction of TAA can be found in the SC in all skin samples even after 24 h incubation. Thus, TAA forms a reservoir in the outermost skin layers from which it is released over a prolonged time period exhibiting a depot effect [49]. This result is consistent with the fact that no TAA could be detected in the acceptor medium.

The solubility parameters of the alkanediols and TAA are similar. However, the Δδ values between TAA and the alkanediols are larger than the difference of the δ values between TAA and SC. Since the solubility of TAA in the SC is already very high, the alkanediols are not expected to have a further significant effect on this factor [64]. Therefore, no effects of the alkanediols can be deduced merely by considering the solubility parameters.

However, it must be considered that the SC is not a homogeneous membrane as it contains both lipophilic and hydrophilic regions. Propylene glycol is known to be inserted into the latter [65,66]. Therefore, it could be hypothesized that 1,2-pentanediol and 2-methyl-2,4-pentanediol are also incorporated into the hydrophilic regions of the lamellar structured SC lipids. As mentioned previously, an earlier study revealed that the release from the tested creams is reduced by the addition of alkanediols [18] while the penetration is increased. Therefore, it is likely that, on the one hand, the alkanediols interact with the SC lipids. On the other hand, as described for propylene glycol, solvation of the keratin of the corneocytes might attribute to the penetration enhancement of the shorter-chained alkanediols [67].

Moreover, some studies suggest that for propylene glycol a solvent drag effect is also responsible for an enhanced drug penetration [50,68]. Additionally, 1,2-pentanediol is suspected to act as a kind of a carrier for the dissolved drug [11]. Lee et al. [51] found alkanediols to be absorbed by the skin. This percutaneous absorption reduces with increasing alkyl chain length. Consequently, 1,2-pentanediol shows the highest penetration [51]. This might indicate that a solvent drag effect additionally contributes to the larger TAA penetration from the 1,2-pentanediol containing formulations.

As alkanediols proved to be surface active, they are predestined to interact with the SC lipids. This alters the barrier function of the SC and consequently affects skin penetration [55]. Therefore, the influence of the tested alkanediols on the content and ordering of SC lipids was examined by means of CRS. While these experiments did not reveal any lipid loss, it became evident that alkanediols increasingly disrupt the lipid order when the alkyl chain length of the alkanediols rises.

Warner et al. found for alkanediols that when exceeding a certain alkyl chain length, a further increase in the number of methylene groups does no longer contribute to an enhanced disordering of SC lipids. 1,2-Hexanediol and 1,2-octanediol intercalate into the lipid bilayer to the same depth [69]. This is in concordance with our results, showing no significant difference between the gauche/trans ratio of 1,2-hexanediol and 1,2-octanediol. However, it should be noted that the aqueous solubility of 1,2-octanediol is 1.82% [70] and its density is lower than that of water. As a result, the 1,2-octanediol excess floats on top of the incubation solution. Therefore, although 5% 1,2-octanediol have been applied the skin is only in contact with a saturated solution.

Thus, the actual concentration applied to the skin is lower than that of the other alkanediols, which dissolve completely in water at a concentration of 5%. Obviously that lower concentration of 1,2-octanediol is still high enough for the intercalation and disordering of SC lipids equally effective as 5% 1,2-hexanediol.

These results indicate that the increased TAA penetration from the formulations with incorporated 1,2-hexanediol and 1,2-octanediol can be explained by disordering the SC lipids. This results in a reduced barrier function of the SC allowing the API to penetrate the SC more easily. As the shorter-chained alkanediols 1,2-pentanediol and 2-methyl-2,4-pentanediol do not substantially affect the lipid order, their penetration enhancement must originate from the effects discussed above.

Differently from the other results, 2-methyl-2,4-pentanediol does not show such a strong penetration-enhancing effect in comparison to the other alkanediols. This alkanediol does not have vicinal hydroxyl groups and therefore has a lower amphiphilicity (data not shown)[4]. As a result, 2-methyl-2,4-pentanediol differs in its properties from the other alkanediols studied. Since the extent of antimicrobial activity of the alkanediols depends on their amphiphilicity, which favors their incorporation into the lipophilic bilayer of the bacterial cell wall, 2-methyl-2,4-pentanediol was found to be less effective in antimicrobial performance than the other alkanediols. In addition, due to the reduced amphiphilicity, the interaction with surfactants and co-surfactants present in the formulation is likewise diminished [4]. In our study, the reduced amphiphilicity leads, on the one hand, to a lower TAA solubility of this compound compared to the other alkanediols [18]. On the other hand, the interaction with the SC components is sterically less favorable.

When comparing the different test formulations, it is noticeable that the penetrated TAA amounts from the gels are generally the highest. For the final evaluation it must be considered that the different formulations had to be assessed using skin samples originating from different donor pigs, as one porcine ear delivers only a limited number of skin punches. However, TAA penetration from the hydrogels is almost double compared to the creams. Moreover, in contrast to the creams TAA release from the hydrogels was much faster than from those [18]. As the penetration experiments were

[4] See Chapter 3, Interfacial Activity of the Alkanediols

performed under finite, non-occlusive conditions, it seems, thus, to be more likely that the comparatively high penetration rate from the hydrogels is associated with the evaporation of water. This causes the TAA concentration on the skin to increase and consequently enhance penetration [71]. Since the hydrogels consist mainly of water, and TAA can be present only in the aqueous phase this effect is here much more pronounced than in the creams.

6.6. Conclusions

In this study, the influence of four different alkanediols on skin penetration of TAA from different semisolid formulations was investigated. The results revealed that the alkanediols tested generally act as penetration enhancers. However, the magnitude of this effect differs depending on the formulation used.

CRS measurements revealed that the studied alkanediols did not lead to an extraction of lipids from SC and consequently the SC thickness was also not altered. However, SC lipids enter a more disordered state as a result of the incubation with the longer-chained alkanediols. This can explain the enhanced TAA penetration when these longer-chained alkanediols are added. In addition, a solvent drag effect, as well as an interaction with the hydrophilic regions of the lamellar ordered SC lipids or the keratin of corneocytes, provides an explanation for the increased penetration from the formulations containing the shorter-chained alkanediols.

2-Methyl-2,4-pentanediol showed only a marginal effect on TAA penetration. This could have been expected as it is not a 1,2-alkanediol. Hence, it has a lower amphiphilicity and its interaction with the SC lipids as well as cell membranes [4] is comparatively lower than those of the other alkanediols.

Overall, alkanediols increased TAA penetration from the formulations tested to a more or less pronounced extent. This should be considered when substituting alkanediols for conventional antimicrobial preservatives. The penetration-enhancing effect depends on both, the respective formulation and the specific alkanediol added. To this end it is unfortunate that it seems almost impossible to predict anything quantitatively due to the extremely high colloidal complexity of the total system comprising cream and skin. Partitioning and solubility of TAA and alkanediols in the diverse compartments/phases of the cream and the skin affects each other and alters also the

structural integrity of the preparations. This might then likewise affect the physical stability and the consistency of the respective formulation as has been shown earlier [4].

Supplementary Materials

Statistical Analysis of TAA skin penetration

Penetrated fraction in stratum corneum, viable epidermis and dermis: two-way ANOVA followed by Tukey's multiple comparisons test

Cumulative skin penetration of triamcinolone acetonide: one-way ANOVA followed by Tukey's multiple comparisons test

The significant differences are presented with a different number of asterisks (*) as follows: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$. ns: not significant.

Aqueous carbomer

a) Penetrated fraction in stratum corneum, viable epidermis and dermis

Stratum corneum	
Reference vs. 2-Methyl-2,4-pentanediol 5 %	*
Reference vs. 1,2-Pentanediol 5 %	****
Reference vs. 1,2-Hexanediol 5 %	****
Reference vs. 1,2-Octanediol 5 %	****
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Pentanediol 5 %	ns
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Hexanediol 5 %	****
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Octanediol 5 %	****
1,2-Pentanediol 5 % vs. 1,2-Hexanediol 5 %	***
1,2-Pentanediol 5 % vs. 1,2-Octanediol 5 %	*
1,2-Hexanediol 5 % vs. 1,2-Octanediol 5 %	ns
Epidermis	
Reference vs. 2-Methyl-2,4-pentanediol 5 %	****
Reference vs. 1,2-Pentanediol 5 %	****
Reference vs. 1,2-Hexanediol 5 %	****
Reference vs. 1,2-Octanediol 5 %	****
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Pentanediol 5 %	ns
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Hexanediol 5 %	ns
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Octanediol 5 %	ns
1,2-Pentanediol 5 % vs. 1,2-Hexanediol 5 %	ns
1,2-Pentanediol 5 % vs. 1,2-Octanediol 5 %	ns
1,2-Hexanediol 5 % vs. 1,2-Octanediol 5 %	ns
Dermis	
Reference vs. 2-Methyl-2,4-pentanediol 5 %	ns
Reference vs. 1,2-Pentanediol 5 %	ns
Reference vs. 1,2-Hexanediol 5 %	ns
Reference vs. 1,2-Octanediol 5 %	*
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Pentanediol 5 %	ns
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Hexanediol 5 %	ns
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Octanediol 5 %	ns

1,2-Pentanediol 5 % vs. 1,2-Hexanediol 5 %	ns
1,2-Pentanediol 5 % vs. 1,2-Octanediol 5 %	ns
1,2-Hexanediol 5 % vs. 1,2-Octanediol 5 %	ns

b) Cumulative skin penetration of triamcinolone acetonide

Reference vs. 2-Methyl-2,4-pentanediol 5 %	***
Reference vs. 1,2-Pentanediol 5 %	****
Reference vs. 1,2-Hexanediol 5 %	****
Reference vs. 1,2-Octanediol 5 %	****
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Pentanediol 5 %	**
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Hexanediol 5 %	**
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Octanediol 5 %	***
1,2-Pentanediol 5 % vs. 1,2-Hexanediol 5 %	ns
1,2-Pentanediol 5 % vs. 1,2-Octanediol 5 %	ns
1,2-Hexanediol 5 % vs. 1,2-Octanediol 5 %	ns

Anionic hydrophilic cream

a) Penetrated fraction in stratum corneum, viable epidermis and dermis

Stratum corneum

Reference vs. 2-Methyl-2,4-pentanediol 5 %	ns
Reference vs. 1,2-Pentanediol 5 %	****
Reference vs. 1,2-Hexanediol 5 %	*
Reference vs. 1,2-Octanediol 5 %	**
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Pentanediol 5 %	****
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Hexanediol 5 %	ns
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Octanediol 5 %	ns
1,2-Pentanediol 5 % vs. 1,2-Hexanediol 5 %	***
1,2-Pentanediol 5 % vs. 1,2-Octanediol 5 %	**
1,2-Hexanediol 5 % vs. 1,2-Octanediol 5 %	ns

Epidermis

Reference vs. 2-Methyl-2,4-pentanediol 5 %	ns
Reference vs. 1,2-Pentanediol 5 %	*
Reference vs. 1,2-Hexanediol 5 %	ns
Reference vs. 1,2-Octanediol 5 %	ns
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Pentanediol 5 %	**
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Hexanediol 5 %	ns
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Octanediol 5 %	ns
1,2-Pentanediol 5 % vs. 1,2-Hexanediol 5 %	ns
1,2-Pentanediol 5 % vs. 1,2-Octanediol 5 %	ns
1,2-Hexanediol 5 % vs. 1,2-Octanediol 5 %	ns

Dermis

Reference vs. 2-Methyl-2,4-pentanediol 5 %	ns
Reference vs. 1,2-Pentanediol 5 %	*
Reference vs. 1,2-Hexanediol 5 %	ns
Reference vs. 1,2-Octanediol 5 %	ns
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Pentanediol 5 %	ns
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Hexanediol 5 %	ns
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Octanediol 5 %	ns
1,2-Pentanediol 5 % vs. 1,2-Hexanediol 5 %	ns
1,2-Pentanediol 5 % vs. 1,2-Octanediol 5 %	ns
1,2-Hexanediol 5 % vs. 1,2-Octanediol 5 %	ns

b) Cumulative skin penetration of triamcinolone acetonide

Reference vs. 2-Methyl-2,4-pentanediol 5 %	**
Reference vs. 1,2-Pentanediol 5 %	****
Reference vs. 1,2-Hexanediol 5 %	****
Reference vs. 1,2-Octanediol 5 %	****
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Pentanediol 5 %	****
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Hexanediol 5 %	*
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Octanediol 5 %	ns
1,2-Pentanediol 5 % vs. 1,2-Hexanediol 5 %	***
1,2-Pentanediol 5 % vs. 1,2-Octanediol 5 %	***
1,2-Hexanediol 5 % vs. 1,2-Octanediol 5 %	ns

Nonionic hydrophilic cream

a) Penetrated fraction in stratum corneum, viable epidermis and dermis

Stratum corneum

Reference vs. 2-Methyl-2,4-pentanediol 5 %	ns
Reference vs. 1,2-Pentanediol 5 %	ns
Reference vs. 1,2-Hexanediol 5 %	**
Reference vs. 1,2-Octanediol 5 %	****
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Pentanediol 5 %	ns
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Hexanediol 5 %	ns
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Octanediol 5 %	***
1,2-Pentanediol 5 % vs. 1,2-Hexanediol 5 %	ns
1,2-Pentanediol 5 % vs. 1,2-Octanediol 5 %	***
1,2-Hexanediol 5 % vs. 1,2-Octanediol 5 %	*

Epidermis

Reference vs. 2-Methyl-2,4-pentanediol 5 %	ns
Reference vs. 1,2-Pentanediol 5 %	ns
Reference vs. 1,2-Hexanediol 5 %	****
Reference vs. 1,2-Octanediol 5 %	****
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Pentanediol 5 %	ns
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Hexanediol 5 %	***
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Octanediol 5 %	***
1,2-Pentanediol 5 % vs. 1,2-Hexanediol 5 %	**
1,2-Pentanediol 5 % vs. 1,2-Octanediol 5 %	**
1,2-Hexanediol 5 % vs. 1,2-Octanediol 5 %	ns

Dermis

Reference vs. 2-Methyl-2,4-pentanediol 5 %	ns
Reference vs. 1,2-Pentanediol 5 %	ns
Reference vs. 1,2-Hexanediol 5 %	*
Reference vs. 1,2-Octanediol 5 %	**
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Pentanediol 5 %	ns
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Hexanediol 5 %	ns
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Octanediol 5 %	ns
1,2-Pentanediol 5 % vs. 1,2-Hexanediol 5 %	ns
1,2-Pentanediol 5 % vs. 1,2-Octanediol 5 %	ns
1,2-Hexanediol 5 % vs. 1,2-Octanediol 5 %	ns

b) Cumulative skin penetration of triamcinolone acetonide

Reference vs. 2-Methyl-2,4-pentanediol 5 %	ns

Reference vs. 1,2-Pentanediol 5 %	*
Reference vs. 1,2-Hexanediol 5 %	***
Reference vs. 1,2-Octanediol 5 %	***
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Pentanediol 5 %	ns
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Hexanediol 5 %	ns
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Octanediol 5 %	*
1,2-Pentanediol 5 % vs. 1,2-Hexanediol 5 %	ns
1,2-Pentanediol 5 % vs. 1,2-Octanediol 5 %	*
1,2-Hexanediol 5 % vs. 1,2-Octanediol 5 %	ns

Basic cream

a) Penetrated fraction in stratum corneum, viable epidermis and dermis

Stratum corneum

Reference vs. 2-Methyl-2,4-pentanediol 5 %	ns
Reference vs. 1,2-Pentanediol 5 %	*
Reference vs. 1,2-Hexanediol 5 %	ns
Reference vs. 1,2-Octanediol 5 %	ns
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Pentanediol 5 %	*
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Hexanediol 5 %	ns
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Octanediol 5 %	ns
1,2-Pentanediol 5 % vs. 1,2-Hexanediol 5 %	***
1,2-Pentanediol 5 % vs. 1,2-Octanediol 5 %	*
1,2-Hexanediol 5 % vs. 1,2-Octanediol 5 %	ns

Epidermis

Reference vs. 2-Methyl-2,4-pentanediol 5 %	ns
Reference vs. 1,2-Pentanediol 5 %	**
Reference vs. 1,2-Hexanediol 5 %	ns
Reference vs. 1,2-Octanediol 5 %	ns
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Pentanediol 5 %	**
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Hexanediol 5 %	ns
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Octanediol 5 %	ns
1,2-Pentanediol 5 % vs. 1,2-Hexanediol 5 %	****
1,2-Pentanediol 5 % vs. 1,2-Octanediol 5 %	ns
1,2-Hexanediol 5 % vs. 1,2-Octanediol 5 %	*

Dermis

Reference vs. 2-Methyl-2,4-pentanediol 5 %	ns
Reference vs. 1,2-Pentanediol 5 %	ns
Reference vs. 1,2-Hexanediol 5 %	ns
Reference vs. 1,2-Octanediol 5 %	ns
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Pentanediol 5 %	ns
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Hexanediol 5 %	ns
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Octanediol 5 %	ns
1,2-Pentanediol 5 % vs. 1,2-Hexanediol 5 %	ns
1,2-Pentanediol 5 % vs. 1,2-Octanediol 5 %	ns
1,2-Hexanediol 5 % vs. 1,2-Octanediol 5 %	ns

b) Cumulative skin penetration of triamcinolone acetonide

Reference vs. 2-Methyl-2,4-pentanediol 5 %	ns
Reference vs. 1,2-Pentanediol 5 %	**
Reference vs. 1,2-Hexanediol 5 %	ns
Reference vs. 1,2-Octanediol 5 %	ns

2-Methyl-2,4-pentanediol 5 % vs. 1,2-Pentanediol 5 %	**
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Hexanediol 5 %	ns
2-Methyl-2,4-pentanediol 5 % vs. 1,2-Octanediol 5 %	ns
1,2-Pentanediol 5 % vs. 1,2-Hexanediol 5 %	**
1,2-Pentanediol 5 % vs. 1,2-Octanediol 5 %	ns
1,2-Hexanediol 5 % vs. 1,2-Octanediol 5 %	ns

Author Contributions

Conceptualization, M.S. and R.D.; methodology, M.S. and R.D.; investigation, M.S.; data curation, M.S.; writing—original draft preparation, M.S.; writing—review and editing, R.D.; visualization, M.S.; supervision, R.D.; project administration, R.D. Both authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

6.7. References

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7. General Conclusion

Alkanediols are widely used as multifunctional excipients in semisolid dermal dosage forms. Particularly, their application as alternative preservatives is of special interest. However, the chemical structure of alkanediols entails the potential for a wide range of interactions both with the formulation and the skin. Therefore, it is the aim of the present thesis to investigate these interactions. In particular, it focusses on the clarification of the four items identified in chapter 2 concerning the application of the alkanediols 2-methyl-2,4-pentanediol, 1,2-pentanediol, 1,2-hexanediol and 1,2-octanediol as alternative preservatives.

As a preparatory work, the interfacial activity of the four alkanediols indicated above was determined in chapter 3 of this thesis. The interfacial activity was found to augment with increasing chain length of the alkyl residue of the substances. Accordingly, this property is directly attributable to the chemical structure of the alkanediols. As a consequence of their hydrophilic and lipophilic parts they exhibit amphiphilic characteristics. 2-Methyl-2,4-pentanediol possesses the lowest interfacial activity due to the different positions of the hydroxyl groups compared to the other three tested alkanediols.

To address the first issue, it is necessary to develop a thorough understanding of the extent to which alkanediols modify the properties of the formulation. To this end, the influence of alkanediols on the stability and the colloidal structure of a nonionic hydrophilic cream was investigated in chapter 4. A longer chain length of the alkanediol yielded a more pronounced incorporation into the lamellar mixed crystals of the cream. This intercalation reduces the consistency of the formulations. However, the consistency of all creams tested remained constant during a six-month storage period, although the consistency diminished as a function of the chain length of the incorporated alkanediol. 2-Methyl-2,4-pentanediol showed the least alteration of the inner structure of the cream, and thus modifies the consistency the least. This behavior originates from the lower amphiphilicity of 2-methyl-2,4-pentanediol compared to the other alkanediols. As discussed in chapter 3, the interfacial activity of this alkanediol is diminished compared to the 1,2-alkanediols.

A test of efficacy of antimicrobial preservation was performed to analyze the preservative effect. This addresses the second item of the list in chapter 2. The results

are presented in chapter 4. The test revealed a dependence of the antimicrobial activity on the length of the alkyl residue of the alkanediols. This correlation is directly related to the amphiphilic structure of the alkanediols and the associated possible incorporation into microbial membranes. A sufficient amount of 1,2-octanediol still remained in the aqueous phase of the cream to ensure adequate preservation, although this substance possesses a limited water solubility as a consequence of its large lipophilic fraction. Thus, 1,2-pentanediol, 1,2-hexanediol and 1,2-octanediol have the potential to substitute conventional preservatives. Only the preservative effect of 2-methyl-2,4-pentanediol alone was not sufficient to pass the preservative challenge test. The reduced preservative effect of the last-named alkanediol is again attributable to its lower amphiphilicity resulting in a weaker incorporation into the microorganisms' cell membranes. Nevertheless, it is feasible to reduce the concentration of a conventional preservative, which was sorbic acid in the present case, when using 2-methyl-2,4-pentanediol.

Chapter 5 reports on whether alkanediols alter the release of the modal drug TAA from various semisolid vehicles. Thus, chapter 5 responds to the third issue of chapter 2. The Higuchi equation could be applied to describe its release behavior, since TAA was present in a suspended state in all four formulations investigated. Alkanediols were found to cause a slight increase of the release of the model drug from the hydrogel, which can be attributed to the increased solubility of TAA in aqueous alkanediol solutions. However, this significantly increased solubility of TAA caused by the addition of alkanediols did not affect the equivalence of the release rates. In contrast, the TAA release rate from the three creams studied declined with the addition of alkanediols, more precisely with increasing length of their alkyl residue. As a consequence, the release rates of the API from two of the studied creams containing alkanediols cannot be considered to be equivalent to that of the pure formulations. These results are consistent with the incorporation of the alkanediols into the mixed crystals of the cream as described above. The magnitude of the effect on the colloidal structure was of the same order of magnitude as the reduction of the TAA release. The effects become more pronounced when the chain length of the alkanediols rises. The declined release rates may be explained by an increased affinity of TAA for the mixed crystals in the presence of the alkanediols.

The modification of the skin penetration and permeation of TAA from the four selected formulations induced by the alkanediols was studied in order to clarify the fourth issue. The results are presented in chapter 6 and reveal that alkanediols generally enhance drug penetration. However, the extent of this effect varies depending on the formulation used. For two creams, 1,2-pentanediol had the strongest penetration-enhancing effect. In contrast to this, the penetration raised with increasing chain length of the alkanediol both for the third cream and the hydrogel. Consequently, 1,2-hexanediol and 1,2-octanediol showed the most pronounced penetration enhancement from the two formulations mentioned last.

Confocal Raman spectroscopy measurements were performed to provide deeper insight into the mechanism of penetration enhancement. The CRS measurements revealed that the studied alkanediols do not result in an extraction of lipids from the SC. Consequently, the addition of alkanediols does not alter the SC thickness. However, the SC lipids shift to a more disordered state as a result of incubation with the longer-chained alkanediols. This may explain the increased TAA penetration from the formulations containing 1,2-hexanediol and 1,2-octanediol. The increased drug penetration from the formulations containing the shorter-chained alkanediols could be attributed to a solvent drag effect as well as an interaction of the alkanediols with the hydrophilic domains of the lamellar ordered SC lipids and the keratin of the corneocytes. 2-Methyl-2,4-pentanediol again differs from the other alkanediols tested in this aspect. Its interaction with the SC lipids is reduced compared to that of the 1,2-alkanediols as a result of its lower amphiphilicity. Thus 2-methly-2,4-pentanediol has only a minor effect on TAA skin penetration.

However, a quantitative prediction of the penetration behavior is hardly possible due to the extremely high colloidal complexity of the overall system which comprises the formulations and skin.

The results of this work reveal that various interactions of these multifunctional ingredients should be considered prior to employing alkanediols as alternative preservatives in dermal products. On the one hand, their preservative effect increases with rising alkyl chain length. On the other hand, the use of longer-chained alkanediols results in an augmented impact on formulation consistency. Furthermore, when using alkanediols in dermal drug products, consideration should be given to their ability to

act as skin penetration enhancers. The extent of this enhancement depends on the respective alkanediol as well as the vehicle applied.

All in all, the results of this thesis clearly demonstrate that alkanediols are very suitable as effective alternative preservatives for semisolid dermal formulations, provided that the above factors are taken into consideration in their application.

8. Appendix

8.1. Influence of Alkanediols on the Rheological Properties of the Aqueous Carbomer Gel

Several carbomer gels were measured by oscillation rheometry using the same method as described in chapter 4.3.4. These gels included the pure ACG without added alkanediols as a reference. The test formulations comprised ACGs with added alkanediols in the following concentrations: 5% 2-methyl-2,4-pentanediol, 5% 1,2-pentanediol, 3% 1,2-hexanediol and 1% 1,2-octanediol, respectively. The formulations were stored at room temperature for a period of six months and measured at 1, 7, 14, 28 days, 3, and 6 months after the preparation.

Both the storage modulus G' in the linear viscoelastic region and the flow point were obtained from the measurements. The storage modulus G’ is regarded as a measure of gel strength. The flow point was determined as the shear stress at the crossover point of the storage modulus G' and the loss modulus G".

Figure 7 displays the storage moduli G’ of the measured ACGs over a period of 6 months. It can clearly be observed that the gel strengths of all tested gels were in the same size range. Although slight variations during the storage period could be identified, all G' values were between 500 and 600 Pa after six-month storage.

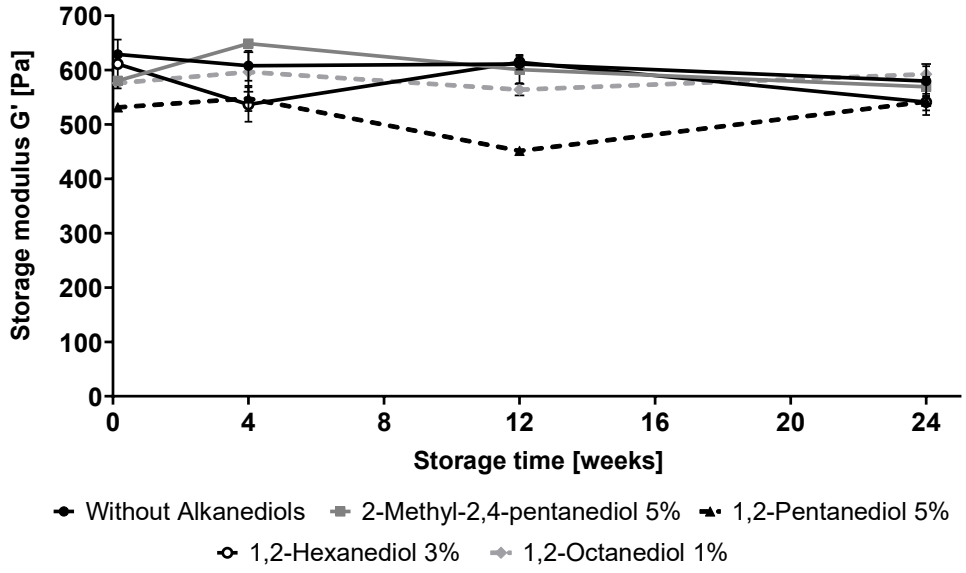

Figure 7. Influence of alkanediols on the storage modulus G’ of ACG, n = 3, mean ± standard deviation.

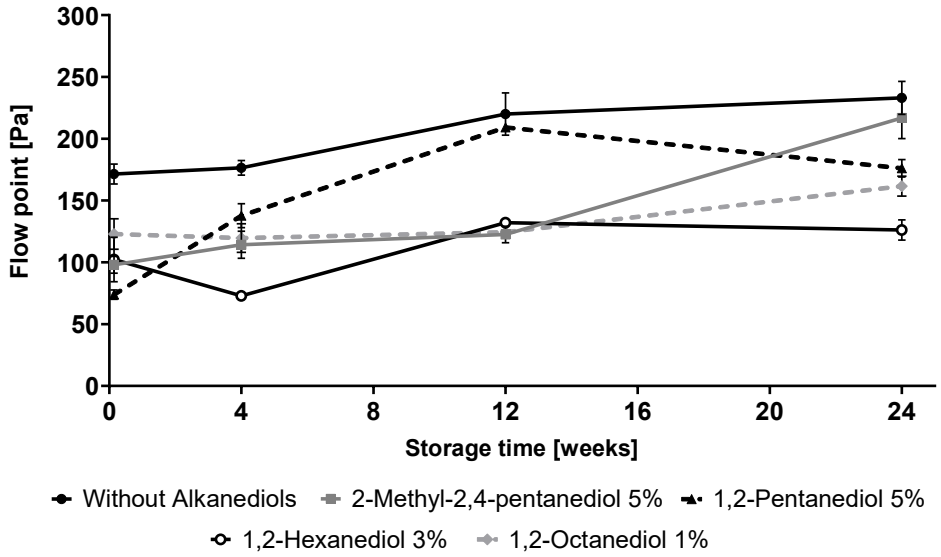

Figure 8. Influence of alkanediols on the flow point of ACG, n = 3, mean ± standard deviation.

The flow points of the different ACGs are represented in Figure 8. These flow points were also in a similar range of magnitude for all gels. There was no clear tendency for the flow point to change as a function of the chain length of the added alkanediols.

Compared to the influence of the alkanediols on the rheological properties of the NHC (chapter 4.4.1), the storage moduli and flow points of the ACGs were not considerably altered when adding alkanediols in the indicated concentrations.

8.2. TAA Solubility in Different Nonionic Hydrophilic Cream Formulations

To study the effect of alkanediols on the solubility of TAA in NHC, alkanediol-containing NHCs with different concentrations of TAA were prepared. These formulations were analyzed for the presence of crystals using an Axio Imager Z1 microscope (Carl Zeiss, Jena, Germany) with crossed polarizers and a ¼ λ-plate.

The appearance of crystals indicates that TAA is not in a fully dissolved state, but rather in an at least partially suspended state. The absence of visible crystals is an indication of complete dissolution of TAA. Except for the reference formulation, all NHCs contained alkanediols in a concentration of 5%. The concentrations of TAA were varied and the obtained results were determined by using the polarization microscope. Based on the outcome, NHCs were subsequently prepared with further, lower or higher, TAA concentrations. The calculated limit of detection for this method is well below the concentrations prepared and analyzed in the present experiment.

Figure 9 shows the results of the examinations, with the crystals marked for better visualization. In the reference NHC, TAA crystals still appeared even at a concentration of 0.002%, indicating that the active ingredient was not completely dissolved. This implies that the saturation solubility of TAA is below 0.002% in the pure NHC.

In the creams containing 2-methyl-2,4-pentanediol, 1,2-pentanediol and 1,2-hexanediol, crystals still appeared at a concentration of 0.006%, while they were not observed at a concentration of 0.004%. From these measurements it can be concluded that the saturation solubility of TAA ranges from 0.004% to 0.006% in these three formulations. Consequently, the incorporation of the three alkanediols indicated above into the NHCs led to an enhancement of the solubility of TAA compared to the NHC without alkanediols.

1,2-Octanediol induced a further increase of the solubility, as TAA dissolved completely at a concentration of 0.02%. Since crystals were detected at a concentration of 0.03%, the saturation solubility is larger than 0.02% but smaller than 0.03%.

From these results it can be concluded that alkanediols cause an increase of the TAA solubility in the NHC. 1,2-Octanediol having the longest alkyl chain of the studied alkanediols led to the greatest increase of the TAA solubility.

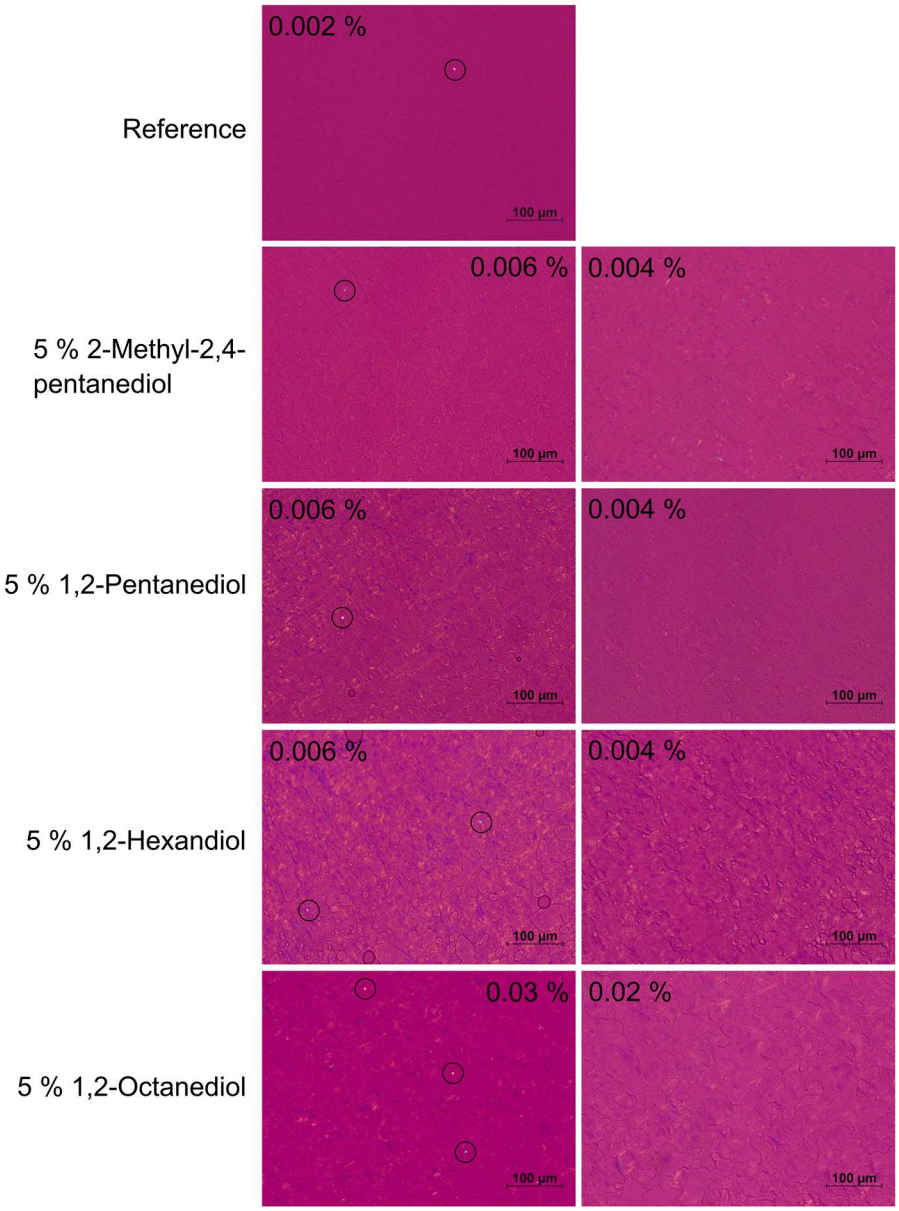

Figure 9. Polarization microscopic images of the tested NHCs containing different amounts of TAA captured with a magnification of 20x. Crystals have been marked for better visualization.