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Abstract: Imiquimod (IMQ) is an immunostimulant drug topically used for the treatment of actinic keratosis and basal cell carcinoma. IMQ formulation and skin delivery is difficult because of its very low solubility in the most of pharmaceutical excipients and very poor skin penetration properties. The purpose of this study was to develop a microemulsion to optimise imiquimod skin delivery using D-α-tocopherol polyethylene glycol-1000 succinate (TPGS) as surfactant (so as to take advantage of its thickening properties) and isostearic acid as oil phase. This fatty acid was selected since it has demonstrated a good solubilizing power for imiquimod and it has also shown to contribute to its therapeutic activity. We have built pseudo-ternary diagrams using two different co-surfactants (Transcutol® and propylene glycol -PG) in a 1:1 ratio with TPGS and then selected microemulsions in the clear and viscous regions of the diagrams. The systems were characterized in terms of rheology and X-ray scattering; additionally, the capability to promote IMQ skin uptake was evaluated ex-vivo on a porcine skin model. All the formulations selected in the gel-microemulsion regions behaved as viscoelastic solids; X-rays scattering experiments revealed in all cases the presence of an ordered lamellar structure, but with differences in terms of interlamellar distance and flexibility between Transcutol® and PG - containing systems. A higher flexibility and a greater hydrophobic volume, possibly interconnected at some point, was associated to the use of Transcutol® and had an impact on the microemulsion capacity to solubilize IMQ as well as on the capability to enhance drug uptake into the skin. The best performing gel-like microemulsion was composed of ≈ 26% of water, ≈21% of isostearic acid, ≈26% of TPGS and ≈27% of Transcutol® and accumulated, after 6h of contact, 3.0 ± 1.1 µg/cm2 of IMQ. This value is higher than the one reported in the literature for the commercial cream (1.9 ± 0.8 µg/cm2), despite the 4-times lower concentration of the vehicle (13 mg/g for the microemulsion vs 50 mg/g for the commercial cream).

***Cover Letter**

Dear Editor,

I am writing to re-submit our manuscript entitled “Microemulsions based on TPGS and isostearic acid for imiquimod formulation and skin delivery “ to be considered for publication in the special issue of the European Journal of Pharmaceutical Sciences dedicated to the 2018 EUFEPS Annual Meeting.

We have revised the manuscript taking into consideration all the comments of the two referee. We have submitted a point-by-point answer and clearly marked the changes in the paper (now in red in the text).

Thank you for your consideration of this manuscript.

Sincerely,

Sara Nicoli

Parma, 05/10/2018

Microemulsions based on TPGS and isostearic acid for imiquimod formulation and skin delivery

Silvia Pescina[1], Gabriela Garrastazu [2], Elena del Favero[3], Valeria Rondelli[3], Laura Cantù[3], Cristina Padula[1], Patrizia Santi[1], Sara Nicoli[1*]

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Keywords: Microemulsion; TPGS; isostearic acid; imiquimod; skin delivery; viscosity; X-ray scattering

ABSTRACT

Imiquimod (IMQ) is an immunostimulant drug topically used for the treatment of actinic keratosis and basal cell carcinoma. IMQ formulation and skin delivery is difficult because of its very low solubility in the most of pharmaceutical excipients and very poor skin penetration properties. The purpose of this study was to develop a microemulsion to optimise imiquimod skin delivery using D-α-tocopherol polyethylene glycol-1000 succinate (TPGS) as surfactant (so as to take advantage of its thickening properties) and isostearic acid as oil phase. This fatty acid was selected since it has demonstrated a good solubilizing power for imiquimod and it has also shown to contribute to its therapeutic activity. We have built pseudo-ternary diagrams using two different co-surfactants (Transcutol® and propylene glycol -PG) in a 1:1 ratio with TPGS and then selected microemulsions in the clear and viscous regions of the diagrams. The systems were characterized in terms of rheology and X-ray scattering; additionally, the capability to promote IMQ skin uptake was evaluated *ex-vivo* on a porcine skin model. All the formulations selected in the gel-microemulsion regions behaved as viscoelastic solids; X-rays scattering experiments revealed in all cases the presence of an ordered lamellar structure, but with differences in terms of interlamellar distance and flexibility between Transcutol® and PG – containing systems. A higher flexibility and a greater hydrophobic volume, possibly interconnected at some point, was associated to the use of Transcutol® and had an impact on the microemulsion capacity to solubilize IMQ as well as on the capability to enhance drug uptake into the skin. The best performing gel-like microemulsion was composed of ≈ 26% of water, ≈21% of isostearic acid, ≈26% of TPGS and ≈27% of Transcutol® and accumulated, after 6h of contact, 3.0 ± 1.1 μg/cm^2 of IMQ. This value is higher than the one reported in the literature for the commercial cream (1.9 ± 0.8 μg/cm^2), despite the 4-times lower concentration of the vehicle (13 mg/g for the microemulsion vs 50 mg/g for the commercial cream).

We thank both the referee for their comments. We have answered to all of them and changed the text accordingly (in red in the manuscript). A point-by-point answer is here reported:	
Reviewer #1: the paper focuses on the design and development of microemulsion based system intended for skin delivery of imiquimod. the paper is interesting and few points should be clarified. general comments	
english should be revised.	**The language has been revised**
the reference products should be better described	**The composition of the reference product has been added to the text (page 19/20)** *"This result is particularly interesting, given the 10-fold different drug concentration (approx. 5 mg/g for the cited MEs vs 50 mg/g for Imunocare®), indicating a much better transport efficiency for the gel-like ME compared to the coarse emulsion (composition: isostearic acid, benzyl alcohol, cetyl alcohol, stearyl alcohol, white soft paraffin, polysorbate 60, sorbitan stearate, glycerol, methyl hydroxybenzoate, propyl hydroxybenzoate, xanthan gum and purified water)."*
the differences of the ex vivo experiments should be better stated also in the experimental part.	**The experimental conditions evaluated during the skin deposition experiments has been clarified in the method section (page 8).** *Formulations evaluated are reported in Table I, additionally, an IMQ saturated solution in isostearic acid was tested. All the donors were applied at infinite dose (200 mg/cm^2, occluded) for 6 hours. In case of PG-containing MEs, two different drug loading were evaluated, with the drug present either in solution or in suspension. The detailed description of the preparation of the donor vehicles used is reported in section 2.9*
could hair influence the drug fate?	**Probably the follicular deposition of the drug can contribute to the results obtained (in particular, the deposition in the dermis can be affected), but –at the moment- this is just speculative, since this aspect has not been investigated here. Indeed, the role of a lamellar structure on drug deposition in the hair follicles it is an interesting topic, worth of further investigation.**
check the acknowledgements section.	**The section has been changed**
Reviewer #2: The author aimed to develop a microemulsion to optimise imiquimod skin delivery using D-<alpha>-tocopherol polyethylene glycol-1000 succinate (TPGS) as surfactant and	

isostearic acid as oil phase. The presence of isostearic acid can increase the stability of the formulation and potentially enhance the therapeutic activity. The presence of TPGS can contribute to enhance drug uptake, confer adequate rheological properties and deliver vitamin E to the skin tissue, possibly mitigating some local reactions. Although the experiment was designed well, there are still a few issues need to be solved before acceptance.	
1. The Pseudo-ternary phase diagram of the Smix/Oil/Water systems using acid as oil phase should be provided in figure 1 to demonstrate the important role of the co-surfactant in the formation of lamellar structures.	**Pseudo-ternary phase diagram obtained using PG and transcutol and oleic acid as oil phase were previoulsy published (Benigni 2018, Telò 2016) and in our opinion it is not appropriate to reproduce it again. However, we agree on the fact that a comparison can be important to underline the role of the different components on the formation of the lamellar structure. For this reason, we have added data on the % of ME and ME gel regions for the 4 different combinations (ISO/PG, ISO/T, OLE/PG, OLE/T). A sentence is now present at page 11** *"The result highlights a relatively large gel-like region when the co-surfactant used was propylene glycol (14% of the diagram area), and a very small one when using Transcutol® (0.1% of the diagram area). A similar trend was previously obtained using oleic acid as oil phase, where the gel-like region decreased from 6% (in case of propylene glycol) to 2 % in case of transcutol (Benigni et al., 2018). This suggests an important role of the co-surfactant structure and/or lipophilicity in the formation of lamellar structures."*
2. The X-ray small angle scattering 2D image of ISO 20 PG should be provided in Fig 2	**The X-ray small angle scattering 2D image of ISO 20 PG has been inserted in Fig 2**
3. The intensity spectra of the SAXS and WAXS region in skin temperature should be provided in Fig 3 to demonstrate the correlation between water content and distances within the aggregated phase at both room and skin temperatures.	**The intensity spectra of the SAXS and WAXS region in skin temperature and the phase swelling behavior have been provided in Fig. 3. The text has been modified accordingly.**
4. The abbreviations of isostearic acid in Table 2 are unaligned with the abbreviations of isostearic acid in Table 1.	**The abbreviations have been corrected and are now homogeneous throughout the manuscript**
5. Imiquimod skin deposition from Oleic acid-saturated solution should be performed.	**This experiment was performed in a previous paper. The value obtained and a comment have been now reported at pag 17.** *"This value is significantly higher than the one previously obtained from a saturated solution in oleic acid (IMQ solubility 73 mg/ml) that resulted 1.62 ± 0.40 µg/cm2*

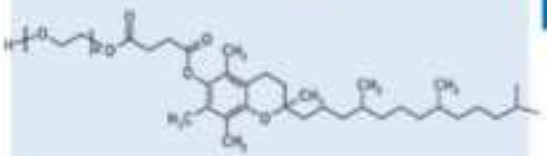
	(Telo et al., 2016b) This result confirms the enhancing property of ISO (Aungst, 1989), in particular toward IMQ permeation (Chollet et al., 1999).
6. The amount of IMT separately found in epidermis and dermis should be presented in Fig 7.	**The data have been moved from the supplementary material to Figure 7**
7. The accumulation value obtained from the commercial cream Imunocare should be presented in Fig 8 to make a comparasion between the Transcutol®-containing microemulsions and the commercial cream Imunocare.	**The data has been added to Figure 8**
8. There is only one Transcutol®-containing microemulsion (ISO 25 T), which is not enough to show a skin deposition higher than the one obtained with the commercial formulation.	**We only evaluated one formulation, because only this composition was able to generate a viscous system (see the very small overlapping area in Figure 1), characteristic that is essential to permit a dermal application. Indeed, as indicated in the conclusion (now red bold) this small design space can represent a problem, and it will be necessary to enlarge it. A possibility could be a change in the surfactant/cosurfactant ratio, but this will be the subject of further studies. The formulation ISO 25 T performed better than imunocare (now in Figure 8), despite the lower drug concentration. This is a good result, even if we agree with the reviewer that the enhancement obtained is not extraordinary.**
9.Some references about TPGS or TPGS based prodrug should be cited.	**References have been added. In particular,** **Collnot et al., 2007** **Zhang et al., 2012** **Muddineti et al., 2017** **Goddeeris et al, 2010**

*Graphical Abstract (for review)

Oil phase: ISOSTEARIC ACID

High stability
High solubilizing power
Therapeutic value

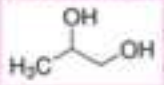
Surfactant: TPGS



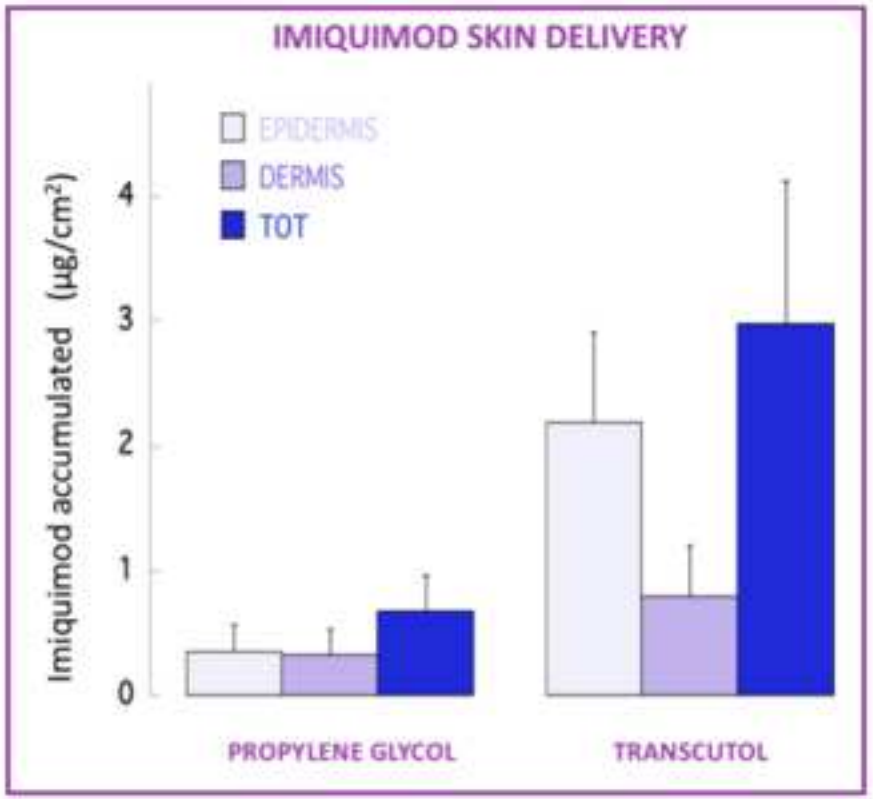
High viscosity systems

Co-surfactant:
either TRANSCUTOL

or PROPYLENE GLYCOL



Influence on system structure and performance



Oil phase	isostearic acid (≈ 21%)	
Aqueous phase	**water (≈26 %)**	
Surfactant	vitamin E TPGS (≈26%)	
Co-surfactant	propylene glycol (≈27%)	Transcutol®(≈27%)

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Keywords: Microemulsion; TPGS; isostearic acid; imiquimod; skin delivery; viscosity; X-ray scattering

ABSTRACT

Imiquimod (IMQ) is an immunostimulant drug topically used for the treatment of actinic keratosis and basal cell carcinoma. IMQ formulation and skin delivery is difficult because of its very low solubility in the most of pharmaceutical excipients and very poor skin penetration properties. The purpose of this study was to develop a microemulsion to optimise imiquimod skin delivery using D-α-tocopherol polyethylene glycol-1000 succinate (TPGS) as surfactant (so as to take advantage of its thickening properties) and isostearic acid as oil phase. This fatty acid was selected since it has demonstrated a good solubilizing power for imiquimod and it has also shown to contribute to its therapeutic activity. We have built pseudo-ternary diagrams using two different co-surfactants (Transcutol® and propylene glycol -PG) in a 1:1 ratio with TPGS and then selected microemulsions in the clear and viscous regions of the diagrams. The systems were characterized in terms of rheology and X-ray scattering; additionally, the capability to promote IMQ skin uptake was evaluated *ex-vivo* on a porcine skin model. All the formulations selected in the gel-microemulsion regions behaved as viscoelastic solids; X-rays scattering experiments revealed in all cases the presence of an ordered lamellar structure, but with differences in terms of interlamellar distance and flexibility between Transcutol® and PG – containing systems. A higher flexibility and a greater hydrophobic volume, possibly interconnected at some point, was associated to the use of Transcutol® and had an impact on the microemulsion capacity to solubilize IMQ as well as on the capability to enhance drug uptake into the skin. The best performing gel-like microemulsion was composed of ≈ 26% of water, ≈21% of isostearic acid, ≈26% of TPGS and ≈27% of Transcutol® and accumulated, after 6h of contact, 3.0 ± 1.1 μg/cm^2 of IMQ. This value is higher than the one reported in the literature for the commercial cream (1.9 ± 0.8 μg/cm^2), despite the 4-times lower concentration of the vehicle (13 mg/g for the microemulsion vs 50 mg/g for the commercial cream).

1. INTRODUCTION

D-α-Tocopheryl polyethylene glycol 1000 succinate (TPGS), is a water-soluble derivative of tocopherol, formed by the esterification of vitamin E succinate with PEG 1000. The presence of a lipophilic tail (tocopheryl succinate) and a hydrophilic head (PEG 1000) confers surfactant properties to this molecule, that has been widely used in pharmaceutical technology and drug delivery for its solubilisation and permeation enhancing properties **(Grimaudo et al., 2018; Guo et al., 2013; Pham and Cho, 2017; Zhang et al., 2012). The capability to inhibit P-glycoprotein mediated efflux (Collnot et al., 2007) has widely promoted its use also in the formulation of antitumor drugs, to overcome anticancer drug resistance (Muddineti et al., 2017).**

Recently, this molecules has been used as a surfactant for the preparation of microemulsions for oral, nasal and dermal administration using different oil phases such as isopropylmiristate (IPM) (Suppasansatorn et al., 2007), oleoyl polyoxyl-6 glycerides (Labrafil® M 1944 CS) (Wan et al., 2017; Yao et al., 2009), omega-3 fatty acids (Lee et al., 2016), propylene glycol monolaurate (Yao et al., 2009), medium-chain triglyceride (Captex® 300) (Ke et al., 2005) and oleic acid (Benigni et al., 2018; Suppasansatorn et al., 2007; Telò et al., 2017). Using oleic acid as oil phase, we have previously reported the possibility to obtain, for specific oil/smix/water ratio, gel-like microemulsions (Benigni et al., 2018). This possibility is particularly interesting in case of dermal application, since the viscosity extends the persistence of the formulation on the application area and, at the same time, enhances patient's compliance (Marty et al., 2005). Even if extensively used for research purposes in ME formulations, oleic acid has some drawbacks, due to the low stability of the oxidable double bond. A possible alternative is represented by isostearic acid (ISO), a mixture of saturated fatty acids consisting mainly of methyl branched isomers of octadecanoic acid. Differently from stearic acid, isostearic acid is liquid at room temperature (melting point<10°C), displays solubilisation characteristics similar to oleic acid, but has higher resistance to oxidation. Its use in semisolid formulations is also supported by toxicological studies, showing lack of skin irritation or sensitization ("4 Final Report on the Safety Assessment of Isostearic Acid," 1983). FDA inactive ingredients database (https://www.accessdata.fda.gov/scripts/cder/iig/index.cfm) indicates a maximum concentration of 25% w/w in topical formulations. Together with these properties, preliminary data suggest the capability of ISO to act as a penetration enhancer (Aungst, 1989) and, in particular, to increase imiquimod skin uptake (Chollet et al., 1999).

Imiquimod (IMQ) is an immunostimulant drug topically used for the treatment of skin and mucosal infections, actinic keratosis and basal cell carcinoma. Its therapeutic effect is mediated by binding to Toll-like receptors 7 and 8, leading to the release of pro-inflammatory cytokines, chemokines and other mediators (Schon and Schon, 2007). IMQ has a very low solubility in many hydrophilic and lipophilic pharmaceutical excipients, but shows good solubility in fatty acids such as isostearic acid (Chollet et al., 1999). Additionally, it has been recently demonstrated that this fatty acid, present as oil phase in the commercial Aldara® cream, has a biological activity and executes additive or synergistic action with the drug (Walter et al., 2013).

The aim of this work was the preparation and the characterization of viscous microemulsions, containing TPGS as surfactant and isostearic acid as oil phase, for imiquimod skin delivery. The presence of isostearic acid can increase the stability of the formulation and potentially enhance the therapeutic activity. The presence of TPGS can enhance drug uptake (Pham and Cho, 2017; Yang et al., 2018), confer adequate rheological properties and deliver vitamin E to the skin tissue, possibly mitigating some local reactions, as reported by Wan et al. (Wan et al., 2017). Indeed, the release of vitamin E from TPGS, in the presence of esterase, has been demonstrated *in vitro* (Grimaudo et al., 2018), and the presence of esterase activity in the stratum corneum is well documented (Beisson et al., 2001; Lau et al., 2012).

In the present work we have 1) evaluated the feasibility of isostearic acid-based gel-like microemulsions using TPGS as surfactant, by building pseudo-ternary diagrams using two different co-surfactants (Transcutol® and propylene glycol); 2) characterized the prepared systems in terms of rheology and mesostructure by X-ray scattering; 3) evaluated the capability of the gel-like systems to promote imiquimod skin uptake using an *in-vitro* skin model.

2. MATERIALS AND METHODS

2.1. Materials

IMQ (MW: 240.3 g/mol; pKa: 7.3) was purchased from Hangzhou Dayangchem, (Zhejiang, China). Oleic acid was purchased from Alfa Aesar (Karlsruhe, Germany) and isostearic acid was a kind gift from Biochim (Milan, Italy). D-α-Tocopheryl polyethylene glycol 1000 succinate (Kolliphor® TPGS, MW: 1513 g/mol) was a kind gift from BASF (Ludwigshafen, Germany) and from ISOCHEM (Gennevilliers, France). Transcutol® was a gift from Gattefossè (Lyon, France). 1,2-propanediol (MW: 76 g/mol) was purchased from A.C.E.F. S.p.A. (Fiorenzuola d'Arda, Italy) while 70% perchloric acid solution, trimethylamine (TEA) and albumin from bovine serum from Sigma Aldrich

(St. Louis, MO, USA). For HPLC analysis, pure water (Purelab® Pulse, Elga Veolia, UK) and HPLC grade acetonitrile and methanol were used.

2.2. Imiquimod quantification method

Imiquimod was quantified by HPLC (Flexar, Perkin Elmer, Waltham, MA, USA), with a reverse-phase C_{18} column (Kinetex C18 2.6 µm, 100 Å, 75 x 4.6 mm, Phenomenex, Torrance, CA, USA), a C_{18} guard column (SecurityGuard Widepore C18, Phenomenex, Torrance, CA, USA) and either UV or fluorescence detection. The mobile phase, pumped at 0.5 ml/min, was a mixture $CH_3OH/CH_3CN/H_2O$/TEA (180/270/530/20). In these conditions, imiquimod retention time was about 4 min. In the case of samples from tissue extraction and permeation experiments, fluorescence detection (λ_{exc} 260 nm, λ_{em} 340 nm) was used (injection volume: 1 µl), while samples used for imiquimod solubility assessment were analysed by UV absorbance (λ 242 nm; injection volume: 10 µl). The HPLC methods were previously validated for sensitivity, precision and accuracy in the concentration intervals 0.03-3 µg/ml for fluorescence detection and 1- 50 µg/ml for UV detection (Telo et al., 2016a).

2.3. Pseudo-ternary phase diagram construction

Pseudo-ternary phase diagrams were built to identify the microemulsion, gel-microemulsion and gel regions in multiphasic systems. Either oleic acid or isostearic acids were used as oil phase, and a 1/1 (w/v; g/ml) mixture of TPGS and co-surfactant (either Transcutol® or 1,2-propanediol) was used as surfactant system (Smix). The diagrams were built using the aqueous tritration method, consisting in the addition of increasing amounts of water (between 5 and 95%) to fixed ratios oil/Smix, namely 0.5/9.5, 1/9, 1.25/8.75, 2/8, 3/7, 4/6, 5/5, 6/4, 7/3, 8/2, 9/1. After each addition, the mixture was vortexed and left 2 minutes to rest, then by visual observation the viscosity and clearness of the system were evaluated. In case of highly viscous systems, the mixture was heated in a water bath at 50°C before each water addition to reduce the viscosity, favour the mixing and achieve homogeneity. The evaluation of the system was performed after cooling at room temperature. The formulation belongs to the microemulsion region if it is clear and exhibits low viscosity, while to the microemulsion-gel region if clear and viscous. The diagrams were built using OriginPro® 2016 (Originlab, Northampton, MA, USA).

2.4. Imiquimod solubility

IMQ solubility was determined in isostearic acid and in the oil/Smix mixtures oleic acid/(TPGS+Transcutol®); isostearic acid/(TPGS+Transcutol®) and isostearic acid/(TPGS+propylene glycole). The oil/Smix ratio was always 3/7, while the surfactant:co-surfactant ratio was always 1:1 (p:v).

Briefly, an excess amount of IMQ was added to the different vehicles, and after 24 hour mixing, the suspension was centrifuged (13000 rpm, 10 minutes). The supernatant was filtered (regenerated cellulose, 0.45µm), diluted and analysed by HPLC-UV for the accurate determination of the solubility.

2.5. Rheological behavior

Rheological data were collected in oscillation mode, using a cone and plate geometry, with an Ares Rheometer (TA Instruments, New Castle, DE, USA) controlled by Orchestrator software (TA instruments, New Castle, DE, USA). Cone (diameter: 50 mm; angle: 0.04 rad) was made of plastic. Sample's linear viscoelastic region (LVE) was determined by strain sweep (10^{-2}-10^{+2} strain %) at 23 °C; dynamic frequency sweep test was then carried out, at the same temperature, at 0.1 % strain for ISO 11 PG and at 0.06% strain for all other samples.

2.6. Polarized optical microscopy

To assess gel-microemulsions optical properties, MEs were spread onto a glass slides and immediately covered with a cover slip to prevent water loss. Samples were analysed at 20X magnification using a polarized optical microscope (Nikon, Shinjuku, Japan) and images were taken with a 13 megapixels camera (Samsung Galaxy S4, Seoul, South Korea).

2.7. X-ray scattering experiments

Small-angle and wide-angle X-ray scattering experiments were carried out to study the internal structure of formulations on length-scales from tens of nanometers down to the tenths of nanometers (Sandri et al., 2017; Telò et al., 2017). Measurements were performed at ESRF Synchrotron (Grenoble, France) on the ID02 high-brilliance beamline in the region of momentum transfer $0.0116 < q < 40$ nm^{-1}, where $q = (4\pi/\lambda) \sin(\theta)$, 2θ is the scattering angle and $\lambda = 0.1$ nm is the incident X-ray wavelength. Samples were measured at 23°C and 33°C, i.e. at normal storage temperature and close to the temperature of the skin. Very short acquisition time was chosen (0.1 s) to avoid any possible radiation damage. 2D intensity patterns were analysed to evidence the

internal organization of different formulations, the presence of intensity rings or arcs indicated ordered internal structures (Cantù et al., 2017). 2D patterns were angularly regrouped to obtain the intensity spectra I(q) and analysed to calculate the structural parameters of each formulation.

All the formulations analysed were blank (without drug) and characterised by a 3/7 oil/Smix ratio. In particular ISO 25 T, its analogue with oleic acid (OLE 25 T), and seven ME containing PG as co-surfactant along the water dilution line (from ISO 11 PG and ISO 25 PG, see Table I and Figure 1b) were evaluated.

2.8. Imiquimod-loaded gel microemulsion

Table I. Composition (% w/w)[a] of the imiquimod-loaded gel-like ME prepared. For all the formulations, the surfactant was TPGS and the oil/Smix ratio was 3/7.

CODE[b]	Oil Phase %		Co-surfactant %		Surfactant (TPGS) %	Water %	IMQ conc (mg/g)	
OLE 25 T	Oleic acid	20.5	Transcutol®	26.7	26.9	25.9	11.8	
ISO 25 T	Isostearic acid	20.5	Transcutol®	26.7	26.9	25.9	13	
ISO 11 PG	Isostearic acid	24.1	propylene glycol	32.9	31.7	11.3	21.2[c]	5.3
ISO 16 PG	Isostearic acid	22.6	propylene glycol	31.1	30.0	16.3	20.0[c]	5.0
ISO 20 PG	Isostearic acid	21.7	propylene glycol	29.5	28.5	20.3	19.1[c]	4.8
ISO 25 PG	Isostearic acid	20.4	propylene glycol	27.5	26.5	25.6	17.8[c]	4.5

[a] *the following densities were used for the calculation: isostearic acid:0.89 g/ml; propylene glycol:1.04 g/ml; Transcutol®:0.99 g/ml*

[b] *The code is given by the oil phase used - Oleic(OLE) or Isostearic (ISO) acid, followed by the water percentage and by the co-surfactant used (T:Transcutol®, PG:propylene glycol)*

[c] *The drug is partially suspended*

In order to obtain IMQ loaded gel-microemulsions, the oil/Smix mixtures (ratio: 3/7) were saturated with IMQ (see section 2.4). Then, known volumes of water were added to obtain the final water % (Table I). The microemulsions were heated at 50°C and vortexed to achieve homogeneity. No precipitation occurred upon water addition in case of OLE 25 T and ISO 25 T. On the contrary, IMQ precipitated from the gelled microemulsions prepared with propylene glycol as co-surfactant, originating white suspensions. The same vehicles were then prepared starting from a 6 mg/ml IMQ solution in oil/Smix mixture.

The composition of the formulations prepared is shown in Table I together with the concentration of imiquimod.

2.9. Stratum corneum (SC) uptake experiments

Epidermis was isolated by soaking full thickness pig ear skin in distilled water at 60°C for 120 s. SC sheets were prepared by soaking isolated epidermis samples in 1% (w/v) trypsine in pH 7.4 PBS, at 4°C for 15 hours. Epidermis was then removed with a cotton swab and SC sheets obtained were carefully rinsed with distilled water, placed on siliconized paper and dried in oven at 37°C for 1 h. The samples were then kept in a dessiccator on $CaCl_2$ until use (Nicoli et al., 2008). For uptake experiments, SC sheets (≈1.6 mg/cm^2, area of approximately 2.5 cm^2) were weighted (Mettler Toledo, sensitivity 0.001 mg) and then individually soaked in 2 ml of isostearic acid solutions containing imiquimod at 0.5, 1.5 or 3 mg/ml concentration. The vials were kept in a temperature-controlled oven at 32±1°C; after 6 h SC sheets were removed from the vehicle, carefully dried using filter paper and re-weighted. Isostearic acid uptake was calculated as:

$$\%\text{Weight increment}=((W_f-W_i)/W_i)\times 100 \qquad \text{Equation 1}$$

where W_f is final weight and W_i is initial weight of SC sheet.

IMQ was then extracted from the SC sheets using 1 ml of oleic acid:methanol mixture (1:3) overnight at room temperature (Telo et al., 2016a).

2.10. Accumulation and permeation experiments

For permeation experiments, porcine skin excised from the outer part of pig ears was used. The skin was separated from the underlying cartilage with a scalpel, frozen at -20°C and used within 3 months. The skin, once thawed, was mounted on glass Franz-type diffusion cells (DISA, Milano, Italy; 0.6 cm^2 surface area) with the stratum corneum facing the donor compartment. The receptor compartment contained 1% w/v albumin solution in PBS pH 7.4 (IMQ solubility: 143 ± 3 µg/ml). **Formulations evaluated are reported in Table I, additionally, an IMQ saturated solution in isostearic acid was tested. All the donors were applied at infinite dose (200 mg/cm^2, occluded) for 6 hours. In case of PG- containing MEs, two different drug loading were evaluated, with the drug present either in solution or in suspension. The detailed description of the preparation of the donor vehicles used is reported in section 2.9.**

At the end of the experiments, the receptor solution was sampled and the donor formulation was carefully removed. The skin surface was then rinsed with distilled water, blotted dry and tape-stripped twice (Scotch Booktape #845, 3M Co., St Paul, MN, USA) to remove possible traces of the formulation. Skin samples were then heated (hairdryer for 60 seconds) and separated into epidermis and dermis with the help of a spatula. IMQ extraction from the tissues was performed

overnight at room temperature using either 1 ml of oleic acid:methanol (1:3) (epidermis) or 1 ml of PEG 400:methanol:HCl 1M (1:2:2) (dermis). To evaluate IMQ permeation, 1 ml of the receptor solution was sampled, added of 50 µl of 70% v/v perchloric acid to precipitate albumin and centrifuged (12000 rpm, 15 minutes). Samples were analysed by HPLC-fluorescence. The extraction procedure was previously validated (Telo et al., 2016a).

3. RESULTS AND DISCUSSION

TPGS is a surfactant showing peculiar properties. Thanks to its ability to form ordered lamellar structures , it has been used to prepare gel-like systems without the need of any thickening agent, using oleic acid as oil phase (Benigni et al., 2018).

3.1. Pseudo-ternary diagram

The first step was the evaluation of the possibility to obtain gel-like microemulsions using isostearic acid as oil phase, to take advantage of the stability of this fatty acid and of its therapeutic contribution (Walter et al., 2013). So, a phase diagram was built using two different co-surfactants, namely Transcutol® and propylene glycol, in a 1:1 ratio with TPGS and isostearic acid (ISO) as oil phase.

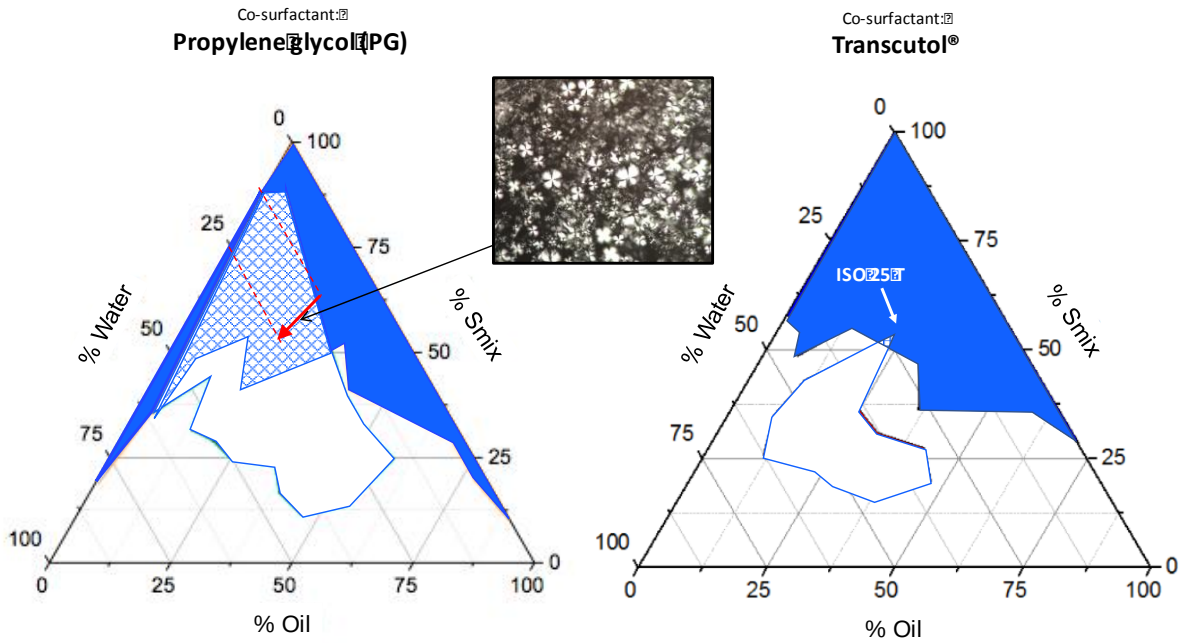

Figure 1: Pseudo-ternary phase diagram of the Smix/Oil/Water systems. The oil phase is isostearic acid, Smix is a mixture of TPGS/co-surfactant 1/1 (w/v). Co-surfactants used are propylene glycol and Transcutol®. The blue region indicates low-viscosity transparent formulations, the white region indicates viscous formulations; the overlapping domains represent clear and highly viscous formulations. In the uncolored region, low viscosity coarse turbid emulsions or phase-separated systems were found. The red arrows in the propylene glycol diagram show the water dilution line investigated (water from 11 to 25%). A representative polarized-light microscope image illustrating the presence of Malta crosses is also shown.

The result highlights a relatively large gel-like region when the co-surfactant used was propylene glycol (14% of the diagram area), and a very small one when using Transcutol® (0.1% of the diagram area). A similar trend was previously obtained using oleic acid as oil phase, where the gel-like region decreased from 6% (in case of propylene glycol) to 2 % in case of transcutol (Benigni et al., 2018). This suggests an important role of the co-surfactant structure and/or lipophilicity in the formation of lamellar structures.

In the clear and viscous regions, some MEs were selected and characterized, since vehicles belonging to this region could – at least in principle - take advantage of the enhancing properties of the ME and the rheological properties of a gel, necessary for a feasible skin application. All the selected ME had an oil/Smix ratio of 3/7, so as to contain a relevant amount of isostearic acid, necessary for boosting the therapeutic efficacy of IMQ (Walter et al., 2013). Their composition is detailed in Table I. In the case of Transcutol®-based ME one formulation was selected, while in case of propylene glycol, different MEs along the 3/7 oil/Smix dilution line (see Figure 1) were chosen, with a water concentration included between 11 and 25% w/w. To evaluate the influence of fatty acid, a ME containing oleic acid as oil phase was also evaluated (OLE 25 T). Its composition (Table I) was the same as ISO 25 T, except for the different fatty acid. The pseudo-ternary diagram related to oleic acid, TPGS and Transcutol® was previously published (Telò et al., 2017), but this ME was never evaluated before.

3.2. Characterization of blank microemulsions

3.2.1. Polarized-light microscopy

The thickening of the microemulsion, obtained for specific oil/Smix/water proportions, is linked to the capability of TPGS to form ordered structures{Goddeeris:2010in}. A preliminary analysis using polarized light microscope highlighted the presence of Malta crosses for all the gel-like microemulsions. The different oil phase (oleic acid *vs* isostearic acid) or co-surfactant (Transcutol® *vs* PG) did not apparently impact on the structure of the system. Figure 1 report a representative image, others are presented in Supplementary material (Figure S1).

3.2.2. X-ray scattering

X-ray scattering experiments were first performed to study the structure of the MEs containing PG as co-surfactant in the range of water content 11-25 %, with an oil/Smix ratio 3/7 (see Figure 1) both at room and skin temperatures (23 and 33 °C).

In the small-angle region (SAXS) the characteristic 2D patterns, as reported in Figure 2 for PG-containing MEs, showed several concentric rings, indicating a well-defined internal structure on the supramolecular length-scale. Interestingly the intensity of each ring was not uniform, rather equatorial arcs were visible for all samples. This peculiar feature was the sign that the formulations consisted of partially aligned structures with characteristic repetitions along the vertical axis. As the samples have been inserted into capillaries (diameter: 2 mm), measured in a horizontal position, these results indicate that formulations, while flowing, assumed a regular internal spacing in the direction perpendicular to insertion. This alignment was more evident at low water content.

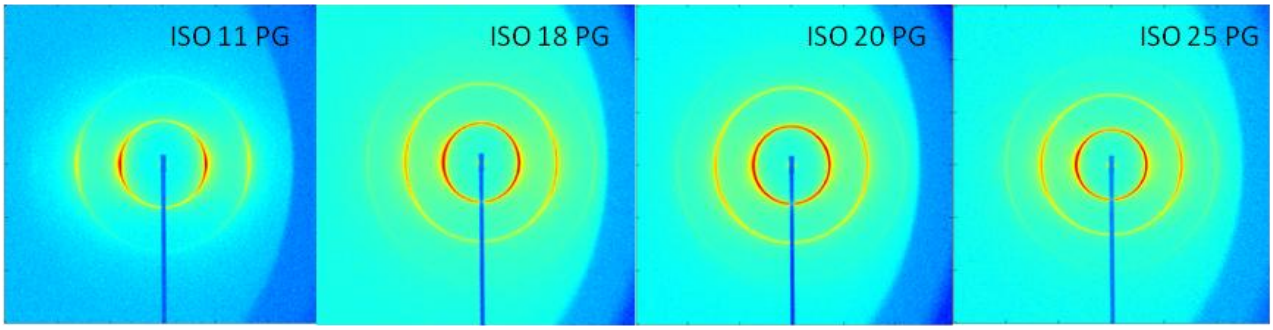

Figure 2. X-ray small angle scattering 2D images relative to ME containing 3/7 oil/Smix ratio, PG as co-surfactant and different water %. From left to right ISO 11 PG, ISO 18 PG, ISO 20 PG, ISO 25 PG, T = 23 °C.

After angular regrouping, each of the scattered intensity profiles presented three peaks with decreasing height (see Figure 3a, T = 23 °C and 3d, T = 33°C). The q position of the subsequent peaks was a multiple of the first one, $q_n = nq_1$, indicating lamellar ordered structures. The inter-lamellar distance was **about 7.6 nm at both temperatures for the ISO 11 PG**, with the lowest water content. Increasing the water content, we observed a left shift of the peaks corresponding to longer characteristic distances within the aggregated phase (d ÷ 1/q) from 7.6 nm to 9.3 nm. In parallel the second and third peaks became higher indicating that the lamellae progressively organized in more ordered structures.

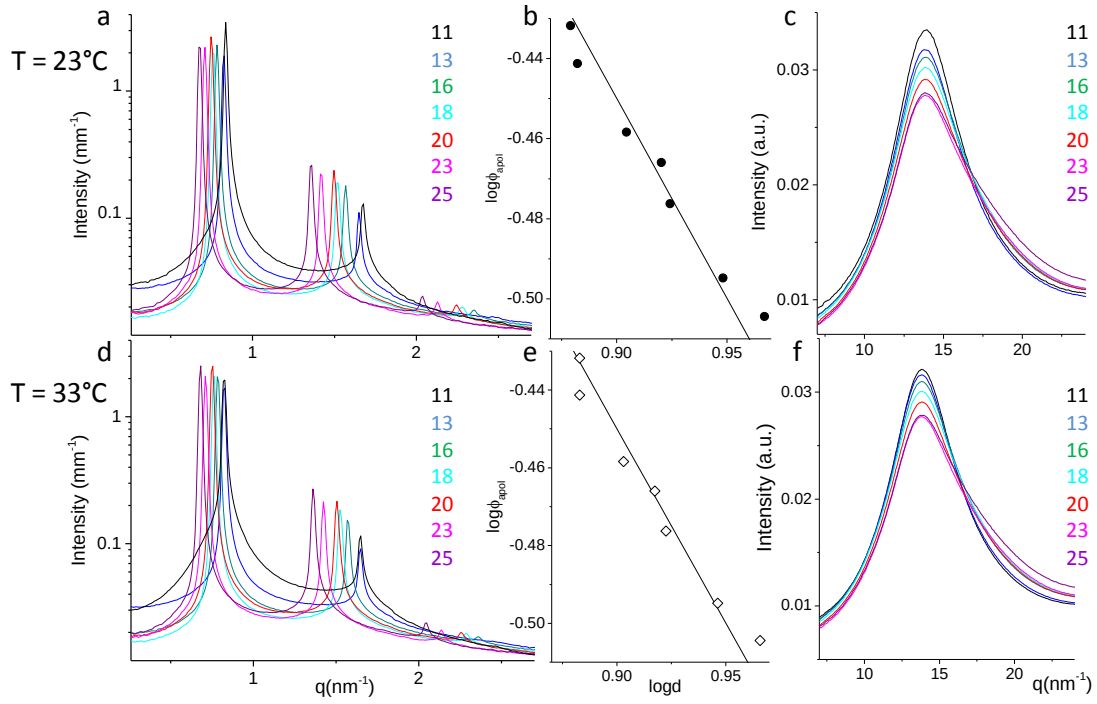

Figure 3. X-ray scattering results relative to ME containing 3/7 oil/Smix ratio, PG as co-surfactant and different water % as indicated in figure at T = 23°C (a, b,c) and at T = 33°C (d,e,f). Panel a,d: SAXS intensity profiles versus q as a function of water content. Panel b,e: Swelling behaviour of MEs. Apolar volume fraction ϕ_{apol} as a function of the characteristic distance d calculated from the first peak position in SAXS intensity profiles $d = 2\pi/q_{peak}$. The line is the best fit obtained from equation $\phi_{apol} \div d^{-s}$ with s = 1. Panel c,f: Plots of WAXS intensity profiles versus q as a function of water content.

The swelling behavior can provide further information on the structural properties of the formulation. In fact, given the general swelling dependence $\phi_{apol} \div d^{-s}$, in which ϕ_{apol} is the apolar volume fraction, the value of the exponent s is connected to the phase of the system, for example, s=1 for the lamellar phase (monodimensional swelling), s=2 for the hexagonal phase (bidimensional swelling), s=3 for the micellar phase (tridimensional swelling). **Figures 3b and 3e report** the apolar volume fraction ϕ_{apol} (calculated as $[(\phi_{isostearic} + \phi_{vitE})]$) as a function of the characteristic distance d for the formulations in the range 11-25% of water content. The distance d was calculated from the position of the first or subsequent peaks $d = 2\pi/(q_{peak}/n)$, where n is the order of the peak, n=1 for the first peak. **The linear fits of the experimental points gave a slope of s=1, characteristic for the swelling behavior of lamellar structures, at both temperatures**. Knowing the volume fraction ϕ_{apol} and the interlamellar distance, we calculated that the thickness

of the apolar layer was about 2.8 nm, enclosed between layers of propylene glycol, polyethylene glycol groups of TPGS and water.

Parallel Wide Angle X-ray Scattering (WAXS) measurements were performed on the same samples to obtain structural information on the very local length-scale, corresponding to the distance between lipid chains in the apolar region. WAXS spectra are reported in Figure 3c (T = 23 °C) **and 3f (T = 33°C).**

The presence of a structure peak at q = 13.9 nm^{-1} (T =23 °C) and q = 13.8 nm^{-1} (T =33 °C) indicated a local order in the lipid region with a mean characteristic distance of d_{local} = 0.452 nm (T =23 °C) and d_{local} = 0.455 nm (T =33 °C). This local order wasn't affected by the addition of increasing amount of water in the investigated formulations.

To elucidate the role of the different excipients on the final structure of MEs, we compared the spectra obtained from ME with the same oil/Smix ratio (3/7), the same water content (25%) but a different co-surfactant or oil phase. Figure 4a reports the intensity spectra in the SAXS and WAXS regions of two ME prepared with isostearic acid and either Transcutol® (orange) or PG (violet) as co-surfactant. On the mesoscale, the results showed a definitely different interlamellar distance: d = 9.2 nm in presence of PG and d = 7.6 nm in presence of Transcutol®. This finding was not unexpected, being the propylene glycol more hydrophilic and hydrated, while Transcutol® could better insert into the oil region.

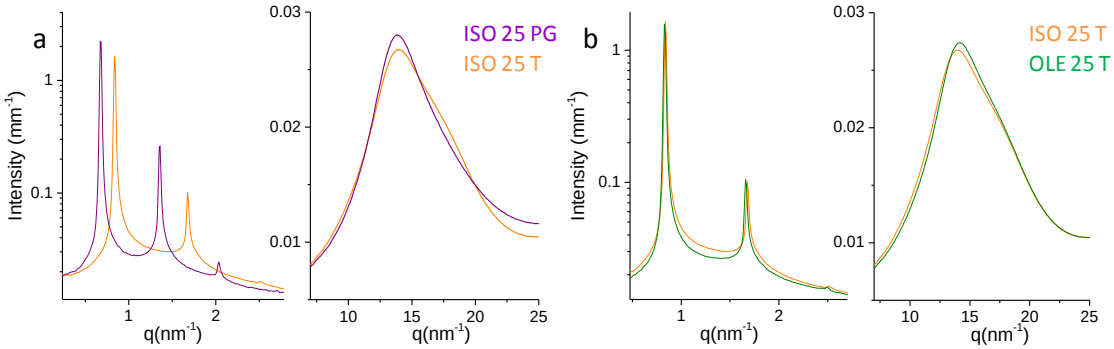

Figure 4. X-ray scattering results relative to ME containing 25% water and 3/7 oil/Smix ratio with different co-surfactants and oils (T = 23°C). Panel a: SAXS (left) and WAXS (right) intensity profiles versus q of ME 25 with isostearic acid and PG co-surfactant (violet) or Transcutol® co-surfactant (orange). Panel b: SAXS (left) and WAXS (right) intensity profiles versus q of ME 25 with Transcutol® co-surfactant and isostearic acid (orange) or oleic acid (green).

In the WAXS region we observed in both samples a first peak centered at the same q = 14 nm^{-1} (d = 0.45 nm), see Figure 4a, but in presence of Transcutol® the intensity spectrum showed also a second peak or shoulder, centered around 16.5 nm^{-1}, corresponding to a shorter characteristic distance d_2 = 0.38 nm. This result indicated that lipid chains underwent a "phase separation" within the single bilayer between regions with closer and looser packing.

Finally, we compared formulations containing Transcutol® as co-surfactant and different oil phases (isostearic or oleic acid) at a given oil/Smix ratio and water content (ISO 25 T and OLE 25 T in Table I). The substitution did not affect the main features of the MEs structures. Similar results on both the mesoscale and the local length-scale were found (Figure 4b), with the characteristic phase separation within the single lamella observed due to the presence of Transcutol®. Moreover, we observed that in presence of Transcutol® both the formulations, with isostearic and oleic acid, did not align internally when they are pushed into capillaries, as the one containing PG (Figure 2), as visible in Fig. S2 (Supplementary material) that shows uniform intensity rings; this means that the systems were able to keep or recover their structure when submitted to confinement and flow. These results, i.e. the presence of uniform intensity rings, the "phase separation" within the single bilayer between regions with closer and looser packing (WAXS), the lower interlamellar distance (7.6 nm) due to Transcutol® surfactant properties, and the absence of the third peak in the SAXS intensity profile (Figure 4a) suggest a more flexible structure in the presence of Transcutol®, that could be associated to the presence of less-organized and connected lamellar structures. On the other hand, the presence of a single peak in WAXS region, the presence of equatorial arcs (Figure 2), the higher interlamellar distance (9.2 nm) and the presence of an evident third peak in the SAXS intensity profile (Figure 4a) suggest, in case of PG, a more rigid and organized structure, characterized by non-interconnected lamellae and a hydrophilic region where the water is engaged by PG and PEG chains. Indeed, as discussed in the following section, PG-containing and Transcutol®-containing systems behave very differently with respect to the capability to maintain imiquimod in solution upon water dilution.

3.2.3. Rheological behaviour

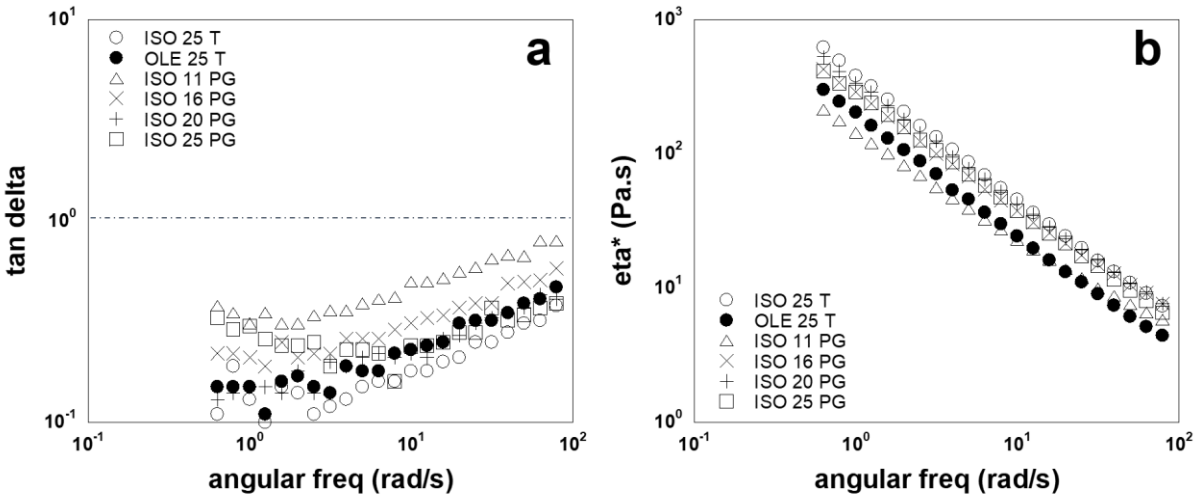

Figure 5. Panel a represents the loss factor (tan delta; G'' and G' ratio): when G'' > G', the loss factor is greater than 1 and material is a viscoelastic liquid; when G" = G', the loss factor is equal to 1 (dashed line), and material show both viscous and elastic behaviour; finally, when G'' < G', the loss factor is lower than 1 (the present case), and sample behaves as viscoelastic solid. Panel b reports the complex viscosity (eta*, Pa.s) against angular frequency (rad/s). The decline of viscosity as consequence of increase of the angular frequency may be translate in term of spreadability, a desirable characteristic for a topical dosage form. Data were collected in oscillatory mode, at 23°C and 0.1% strain for ISO 11 PG and at 0.06% strain for all other samples, using a cone and plate geometry.

All microemulsions behave as viscoelastic solid, with a strong gel structure. In fact, G' (i.e. the storage modulus) is higher than G" (i.e. the loss modulus), as gathered from Figure 5a, where the loss factor (tan delta, i.e. G'' and G' ratio) is always lower than 1 (see also Supplementary material, where Figure S3, reports the rheological profiles of the single MEs). Differences in oil phase (isostearic acid *vs* oleic acid), slightly affected the rheological profile, as shown in Figure 5b. In fact, the complex viscosity (eta*; Pa.s) in presence of oleic acid (OLE 25 T) is lower than that of ME containing isostearic acid (ISO 25 T). This result is not affected by co-surfactant, since when Transcutol® is replaced by PG (ISO 25 T *vs* ISO 25 PG), no difference in complex viscosity is observable. Finally, ISO 11 PG, the ME containing the smaller percentage of water, exhibits lower complex viscosity values, if compared to ISO 16-20-25 PG and, indeed, it is located at the edge of the gel like region in the pseudo-ternary diagram.

Rheological properties correlated with structural ones. At low water content (ISO 11 PG), where we observed the lowest complex viscosity, ME became pretty aligned internally while flowing into capillaries, as reported in Figure 2. This alignment was less evident, but still detectable at higher water content and could be connected to the decrease of the shear viscosity on increasing the shear rate, as inferable from the complex viscosity behaviour against angular frequency reported

in Figure 5b. These results can give interesting insights in view of the development of formulations for topical applications with the desired spreadability.

3.3. IMQ solubility and loading into the MEs

Imiquimod solubility was evaluated in isostearic acid and in the 3/7 oil/Smix mixtures. The results (Table II) are compared with the solubility obtained in a previous paper (Telo et al., 2016a) with oleic acid, pure Transcutol® and pure propylene glycol. The data show the 2 fold higher solubility of IMQ in isostearic acid with respect to oleic acid. The addition of the mixture TPGS:Transcutol® (1:1) to obtain an oil/Smix ratio of 3/7, drastically reduced IMQ solubility to *approx.* 15 mg/ml, regardless the fatty acid contained. When using propylene glycol instead of Transcutol®, drug solubility in the oil/Smix increased, despite the IMQ solubility in the pure co-solvent was double for Transcutol® with respect to PG.

Table II. Imiquimod solubility in the oil phases, co-surfactants and oil/Smix 3/7 mixtures

Vehicle	Solubility (mg/ml)
ISO	154 ± 0.85
OLE	73.86 ±14.2*
Propylene glycol	0.60 ±0.03*
Transcutol®	1.11 ±0.07*
OLE/TPGS/Transcutol® (3/4.5/4.5)	13.40 ± 1.28
ISO/TPGS/Transcutol® (3/4.5/4.5)	16.21 ± 0.13
ISO/TPGS/propylene glycol (3/4.5/4.5)	23.93 ± 2.83

** From ref.*(Telo et al., 2016a)

In order to prepare the gel-like systems, water was added to the saturated oil/Smix (3/7 ratio) solution. Due to the very low aqueous solubility, the addition of water to the isostearic acid/TPGS/PG saturated mixture caused drug precipitation and the formation of a white, gel-like suspension. To avoid this phenomenon, imiquimod was also dissolved in the isostearic acid /TPGS/PG mixture at 6 mg/ml concentration. Upon water addition, ISO 11 PG, ISO 16 PG and ISO 20 PG were transparent, while a slight opalescence was present in case of ISO 25 PG.

Contrarily to the behaviour seen with PG, the addition of water up to 25% to the vehicles containing Transcutol® (OLE 25 T and **ISO** 25 T) did not cause any drug precipitation, suggesting that the more flexible mesostructure, characterized by a greater hydrophobic volume, possibly interconnected at some point, allows a more efficient loading of the drug, at the same time

preserving it from the unfavorable contact with the aqueous phase. The concentration of the IMQ-loaded MEs obtained and further evaluated is reported in Table 1.

3.4. Imiquimod skin deposition from isostearic acid-saturated solution

At first, a saturated solution of imiquimod in pure **ISO** (154 ± 0.85 µg/ml) was applied to the skin tissue for 6 h. The amount accumulated was very high, being 22.27 ± 8.24 µg/cm^2. **This value is significantly higher than the one previously obtained from a saturated solution in oleic acid (IMQ solubility 73 mg/ml) that resulted 1.62 ± 0.40 µg/cm^2 (Telo et al., 2016b) This result confirms the enhancing property of ISO (Aungst, 1989), in particular toward IMQ permeation (Chollet et al., 1999).** The drug remained mainly localized in the epidermis (18.38 ± 9.1 µg/cm^2) and only about 17% was present in the dermis (3.88 ± 2.24 µg/cm^2). This data suggests the presence of an important solvent drag effect, i.e. the penetration of IMQ-saturated isostearic acid in the SC and a slower/limited diffusion of the drug into the underlying tissues, due to the low diffusivity of **ISO** in the hydrophilic derma (Telo et al., 2016a).

Indeed, when stratum corneum sheets were immersed in a solution of imiquimod in isostearic acid, the increase of SC weight (due to isostearic acid uptake) was 26.0 ± 6.5% and the uptake of drug into the SC was linearly correlated with the solvent uptake (Figure 6).

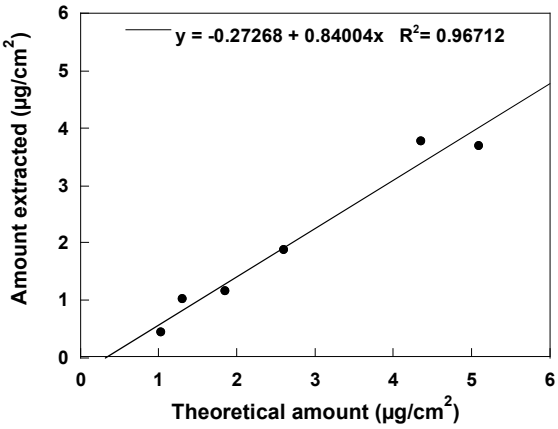

Figure 6. Correlation between the amount of IMQ extracted from the SC and the theoretical amount calculated considering the solvent uptake (ml/cm^2) and the IMQ concentration in the solution (µg/ml)

3.5. Imiquimod skin deposition from microemulsions

Imiquimod was never found in the receptor compartment.

3.5.1. PG-containing microemulsions

4 microemulsions containing PG were selected on the 3/7 oil/Smix dilution line (Figure 1, Table I). Figure 7 show the skin accumulation obtained from these MEs where IMQ was dissolved.

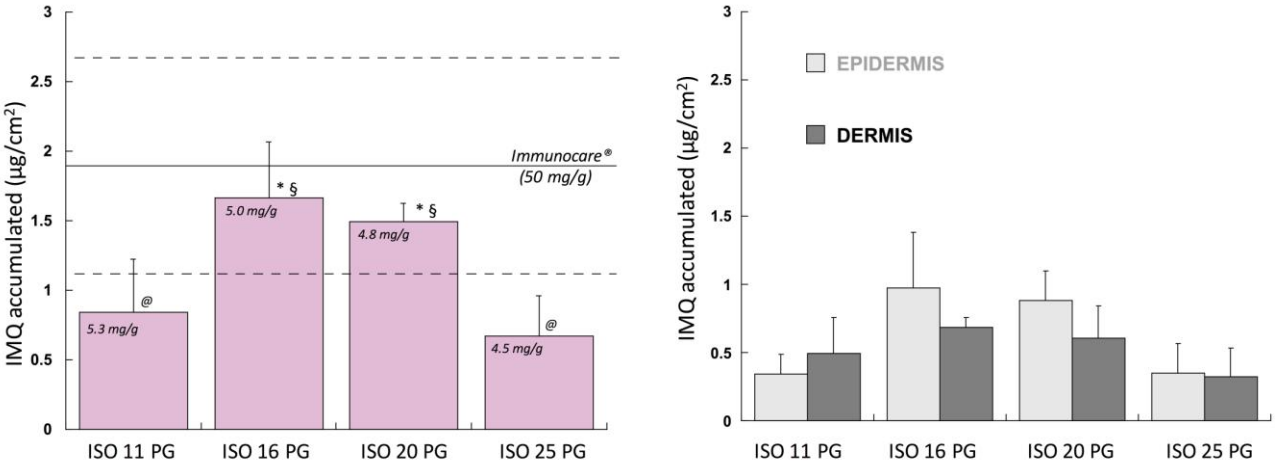

Figure 7. IMQ skin retention (µg/cm^2; mean ± sd) in porcine skin (epidermis+dermis) from microemulsions composed of isostearic acid, TPGS and propylene glycol with increasing water content. All the MEs have a 3/7 oil/Smix ratio, the exact composition is presented in Table I. The horizontal lines represent the accumulation values obtained from the commercial cream Imunocare® (Telo et al., 2016a) (mean ± sd). Symbols indicate that IMQ skin levels are statistically different ($p<0.05$) from ISO 11 PG (*); ISO 25 PG (§); Imunocare®(@). Panels b report the values separately obtained in epidermis and dermis

The result in Figure 7 (IMQ dissolved) highlight that the water % in the system influenced skin uptake. Indeed, when water content was either 16 or 20%, the deposition was statistically higher for comparison with 11 and 25%. The reason is not known but could be attributed to an ideal balance, obtained for a 16-20% water content, between skin hydration (likely increasing with ME water content) and system flexibility: by increasing water content the order within the lamellar phase propagated to longer distances, as described in the structural results, possibly reducing drug mobility. Moreover, the unfavorable hydrophilic layers between drug-loaded lamellae became thicker and thus more difficult to cross. The horizontal line in the figure refers to the IMQ skin deposition obtained with the commercial formulation Imunocare® (Telo et al., 2016a), an Aldara® equivalent, that resulted similar to ISO 16 PG and ISO 20 PG. This result is particularly interesting, given the 10-fold different drug concentration (*approx.* 5 mg/g for the cited MEs *vs* 50 mg/g for Imunocare®), and indicates a much better transport efficiency for the gel-like ME **compared to the coarse emulsion (composition: isostearic acid, benzyl alcohol, cetyl alcohol, stearyl alcohol, white soft paraffin, polysorbate 60, sorbitan stearate, glycerol, methyl hydroxybenzoate, propyl hydroxybenzoate, xanthan gum and purified water).**

To evaluate the possibility to increase IMQ skin accumulation, the same ME containing suspended IMQ (see section 3.3) were evaluated and a 2-4 times higher uptake was found. However, due to

the high variability, the increase is statistically significant only for ISO 11 PG and ISO 25 PG, and it is mainly due to an increase in epidermis accumulation (Supplementary Material, Figure S4). This result could be due to the presence of small IMQ particles trapped in the deep skin farrows that were not removed by the tape stripping procedure used for skin cleaning.

3.5.2. Transcutol®-containing microemulsions

When Transcutol®-based systems were evaluated, a higher accumulation was found (Figure 8). A possible reason is linked to the higher concentration of the vehicle, but it is important to consider that all of them are saturated, thus characterized by the same thermodynamic activity that is the driving force for drug diffusion. More probably, the different mesostructure of Transcutol®-based systems (Figure 4a), more flexible compared to PG-based systems, increased IMQ diffusivity into the vehicle and favoured IMQ-skin interaction. Indeed, the skin levels obtained with ISO 25 T are more than 4 times higher with respect to ISO 25 PG ($p<0.005$). It is also worth underlying that skin accumulation from ISO 25 T is also statistically higher with respect to Imunocare® ($p<0.05$). The better performance of Transcutol®-containing ME with respect to PG-containing ME was also found in case of cyclosporine skin delivery (Benigni et al., 2018), even if with a different oil phase and oil/Smix ratio.

The use of oleic acid instead of isostearic acid, slightly reduced the uptake, but the difference was not statistically significant, in agreement with the comparable mesostructure (Figure 4b).

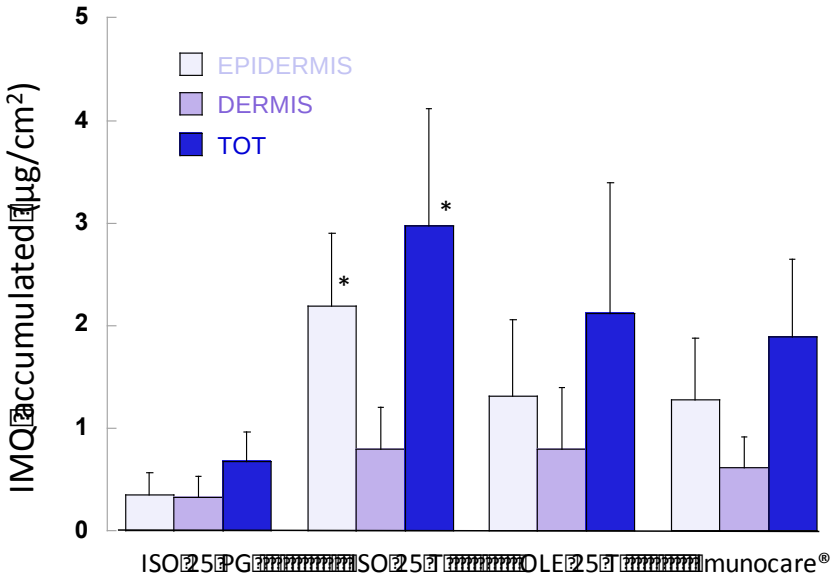

***Figure 8**. IMQ skin deposition (mean±sd) in epidermis, dermis and in the whole skin, from microemulsions containing 25% water and different oil phase acid (oleic or isostearic acid) and co-surfactant (Transcutol® and PG). The exact composition of the vehicles is reported in Table I. * Statistically different from ISO25 PG (p<0.005) **and from Imunocare® (p<0.05). The Imunocare ® data are from ref** (Telo et al., 2016b)*

4. Conclusion

In the present paper, viscous microemulsions based on isostearic acid and TPGS were prepared, characterized and used to deliver imiquimod to the skin. The result obtained show a skin deposition higher than the one obtained with the commercial formulation, despite the lower drug loading. The result also permit to infer the important role of the co-surfactant in determining the microemulsion structure and, as a result, the ME performance.

The formulation ISO 25 T represents a promising vehicle for imiquimod skin delivery; its composition could take advantage of the biologic activity of isostearic acid, the thickening properties of TPGS (and potentially its antioxidant power), and the flexibility imparted by Transcutol® and necessary for an efficient drug deposition into the skin. **However, the very limited size of the gel-like ME region in the pseudo-ternary diagram is a limitation. This extremely restricted "design space" can make small formulation changes very critical. For this reason, it will be necessary to optimize this formulation to enlarge the gel-like ME area, for instance by modifying the surfactant-co-surfactant ratio.**

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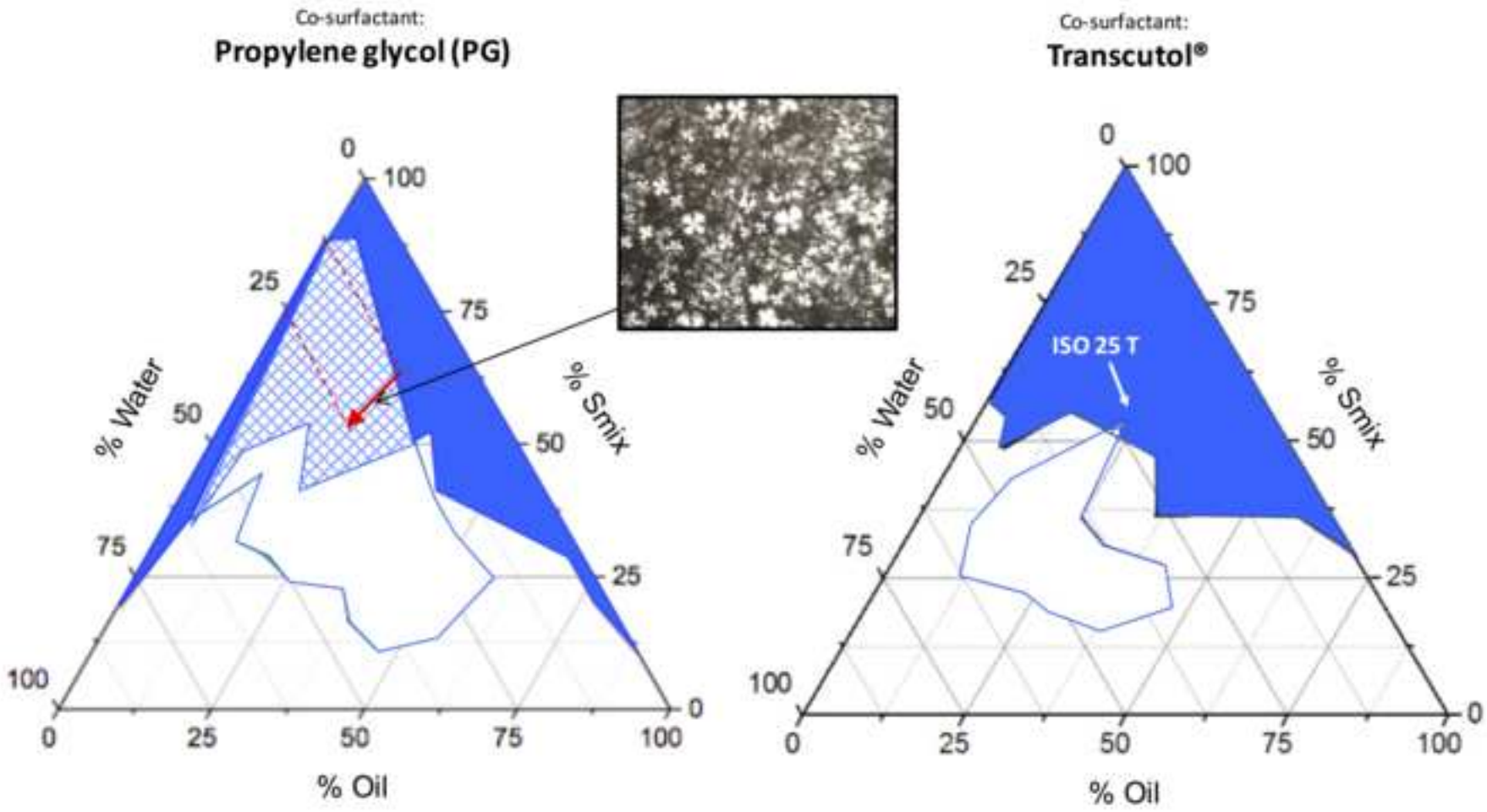

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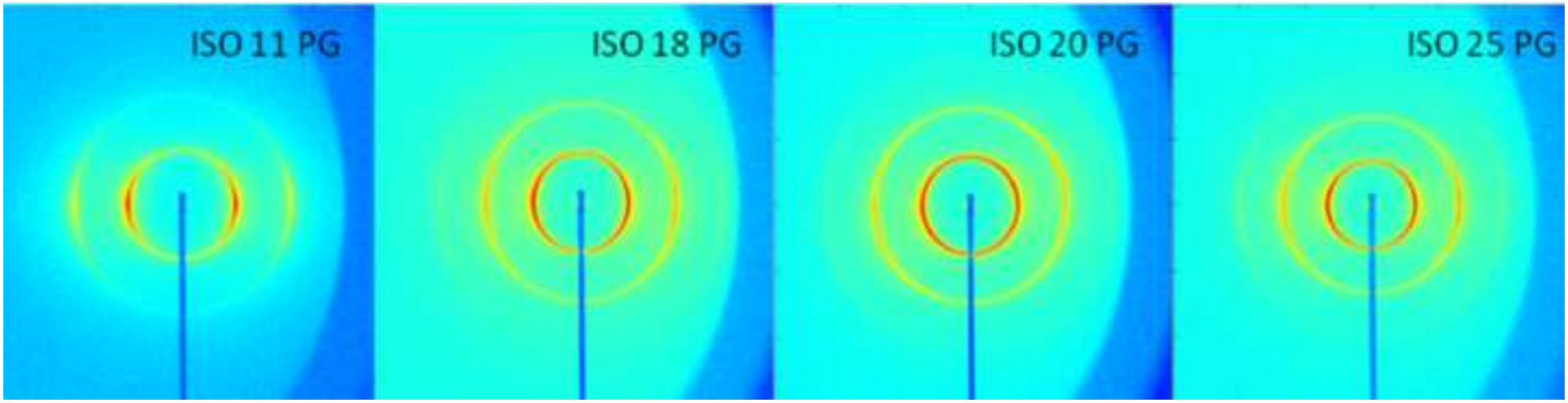

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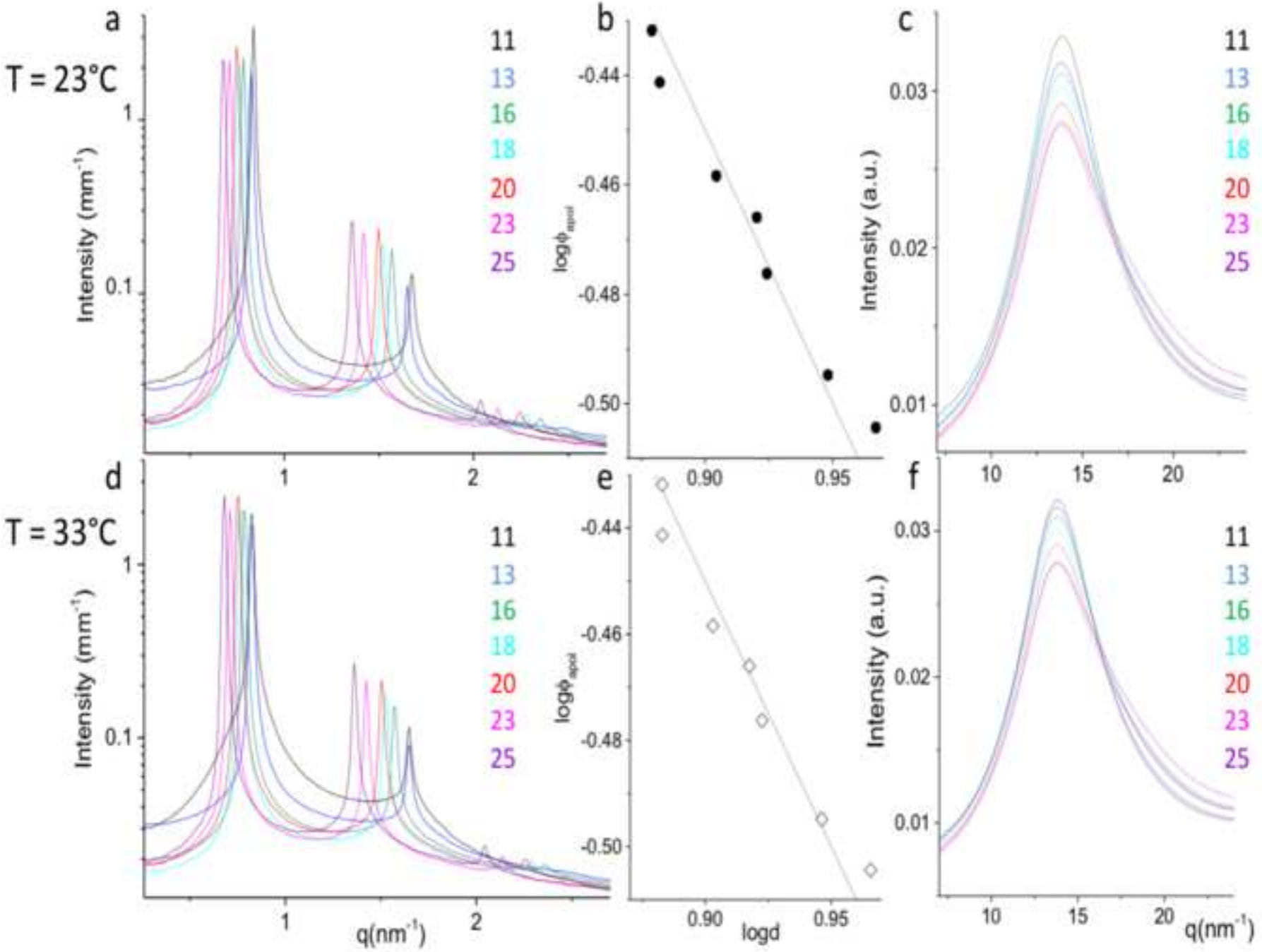

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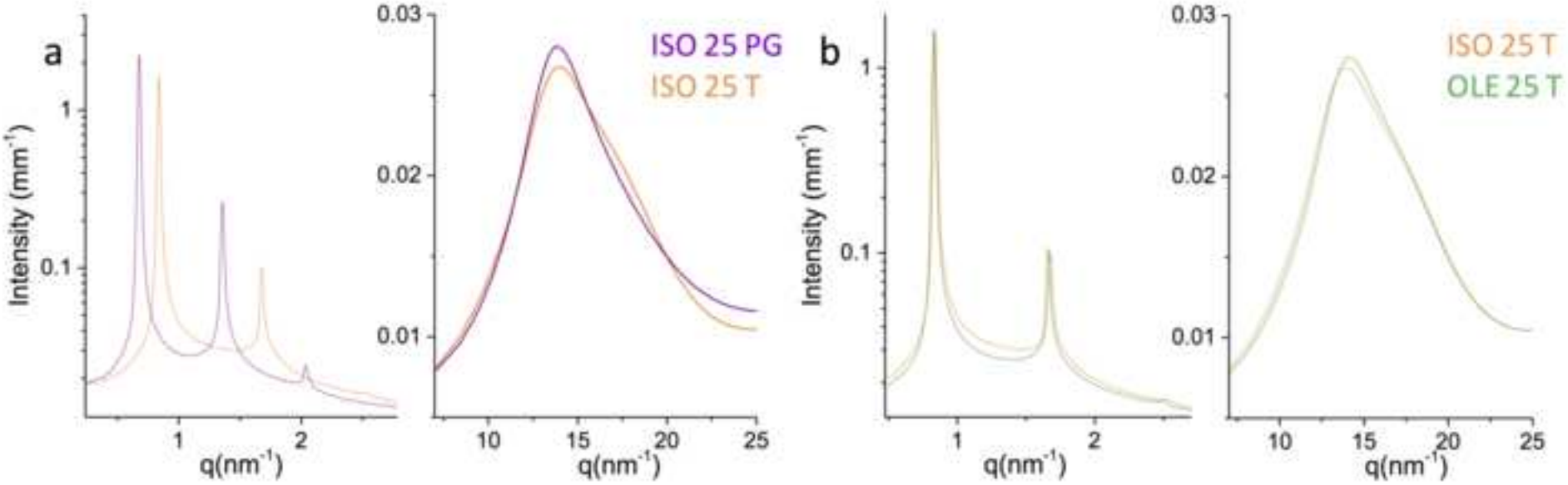

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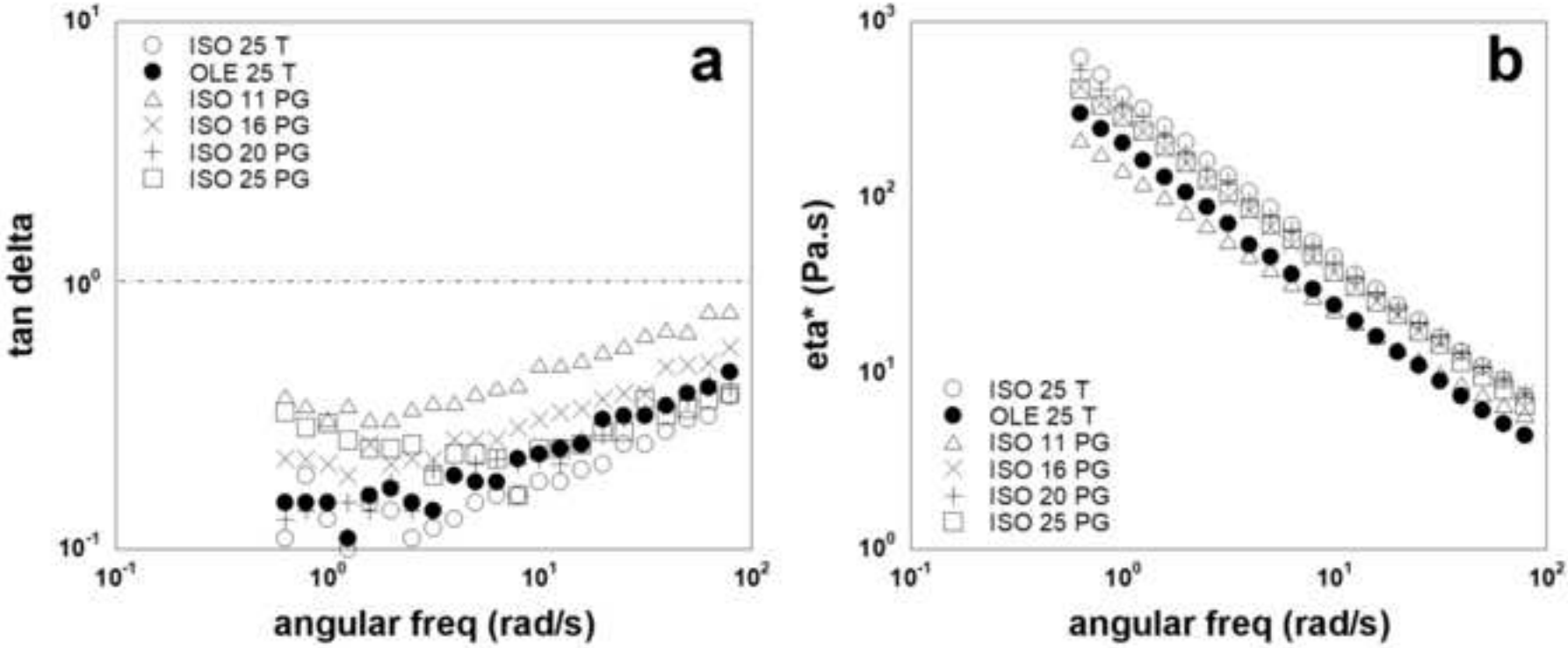

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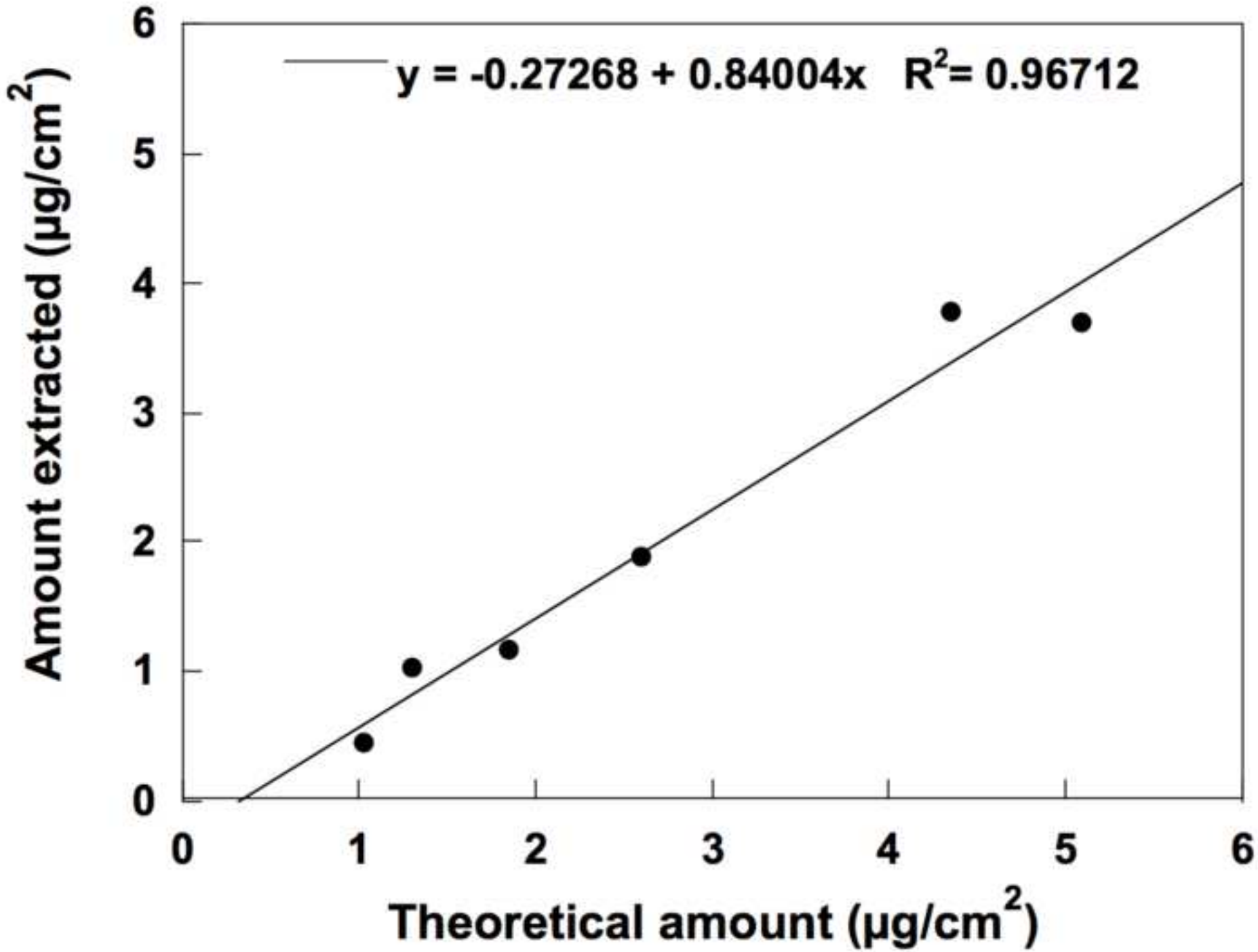

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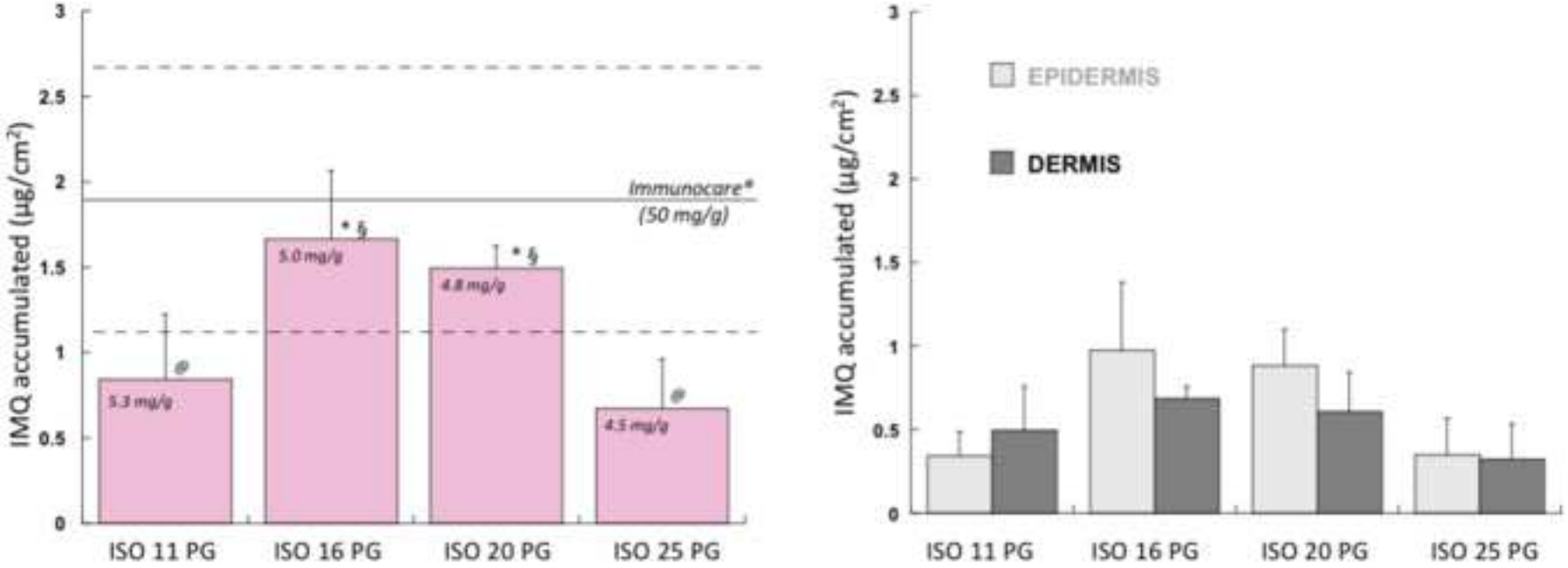

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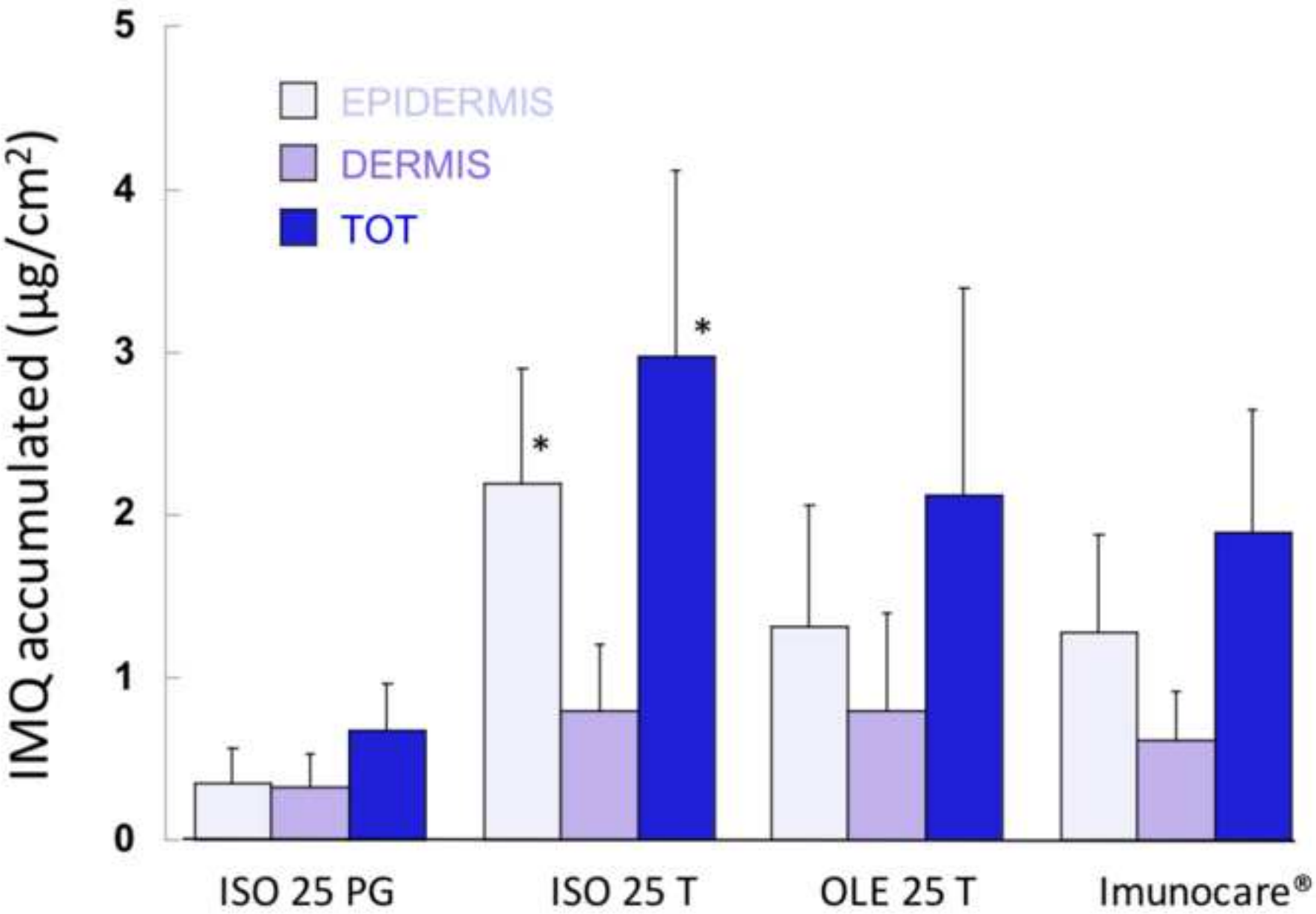

Table 1

Table I. Composition (% w/w)[a] of the imiquimod-loaded gel-like ME prepared. For all the formulations, the surfactant was TPGS and the oil/Smix ratio was 3/7.

CODE[b]	Oil Phase %		Co-surfactant %		Surfactant (TPGS) %	Water %	IMQ conc (mg/g)	
OLE 25 T	Oleic acid	20.5	Transcutol®	26.7	26.9	25.9	11.8	
ISO 25 T	Isostearic acid	20.5	Transcutol®	26.7	26.9	25.9	13	
ISO 11 PG	Isostearic acid	24.1	propylene glycol	32.9	31.7	11.3	21.2[c]	5.3
ISO 16 PG	Isostearic acid	22.6	propylene glycol	31.1	30.0	16.3	20.0[c]	5.0
ISO 20 PG	Isostearic acid	21.7	propylene glycol	29.5	28.5	20.3	19.1[c]	4.8
ISO 25 PG	Isostearic acid	20.4	propylene glycol	27.5	26.5	25.6	17.8[c]	4.5

[a] *the following densities were used for the calculation: isostearic acid:0.89 g/ml; propylene glycol:1.04 g/ml; Transcutol®:0.99 g/ml*

[b] *The code is given by the oil phase used - Oleic(OLE) or Isostearic (ISO) acid, followed by the water percentage and by the co-surfactant used (T:Transcutol®, PG:propylene glycol)*

[c] *The drug is partially suspended*

Table II

Table II. Imiquimod solubility in the oil phases, co-surfactants and oil/Smix 3/7 mixtures

Vehicle	Solubility (mg/ml)
ISA	154 ± 0.85
OLE	73.86 ±14.2*
Propylene glycol	0.60 ±0.03*
Transcutol®	1.11 ±0.07*
OLE/TPGS/Transcutol® (3/4.5/4.5)	13.40 ± 1.28
ISA/TPGS/Transcutol® (3/4.5/4.5)	16.21 ± 0.13
ISA/TPGS/propylene glycol (3/4.5/4.5)	23.93 ± 2.83

** From ref.*(Telo et al., 2016)

Supplementary Material

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