The effect of the presence of biosurfactant on the permeation of pharmaceutical compounds through silicone membrane

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Abstract

The permeation of ten model drugs through silicone membrane was analysed to investigate the effect of the presence of a biosurfactant obtained from corn steep liquor. The ten selected pharmaceutical compounds were chosen to include a diverse range of physicochemical properties, such as variable hydrophobicities, pKa's, molecular masses and degrees of ionisation. When compared with compound permeation alone, the additional inclusion of biosurfactant in the donor phase altered the rate and extent of permeation. It significantly enhanced permeation for five of the compounds, whereas it decreased permeation for four of the compounds and remained approximately the same for the tenth compound. These effects were observed at both biosurfactant concentrations considered, namely 0.005 mg/mL, i.e. below the critical micellar concentration (CMC) and 0.500 mg/mL, i.e. above the CMC of the biosurfactant. Upon analysing permeation change with respect to physicochemical properties of the compounds, it was determined that compounds with a relative molecular mass below 200 resulted in an increase in permeation with biosurfactant present, and those above 200 resulted in a decrease in permeation with biosurfactant present. This effect was therefore attributed to the formation of a drug-biosurfactant interaction that enhanced permeation of smaller compounds, yet retarded permeation for those with a higher molecular mass. These *in vitro* findings can be considered an indication of potential novel formulation options that incorporate biosurfactant to create transdermal products that have bespoke permeation profiles.

Keywords: PDMS; biosurfactant; permeation; membrane; polydimethylsiloxane; flow-through diffusion.

Statistical Summary: words = 5045, tables = 4, figures = 3 (+1 supp)

Introduction

Biosurfactants are natural detergents composed of lipids, sugars and/or proteins [1]. They are known for being more biocompatible than their chemical homologues, such as sodium dodecyl sulphate (SDS), themselves used in pharmaceutical formulations to solubilise active ingredients and improve permeation through skin.

Biosurfactants display similar properties to the more well-known standard surfactants. However, they are produced by microorganisms, and composed of biomolecules, being less toxic, more biocompatible, and biodegradable than chemical surfactants [2]. Many studies have demonstrated the potential uses of biosurfactants in different fields, including environmental applications [3, 4], the food industry [5, 6] and cosmetic formulations [7, 8]. However, very limited research exists regarding the application of biosurfactants in the pharmaceutical industry. Related to the applications of biosurfactants in these two last areas, some researchers have observed an antimicrobial activity of several biosurfactants against pathogenic strains [9-11]. Additionally, researchers have discovered that a biosurfactant obtained from corn steep liquor (CSL) exhibits sunscreen protective properties [12]. Corn steep liquor is a corn stream, obtained from the corn wet-milling industry, spontaneously fermented by lactic acid bacteria, which is "Generally Recognized As Safe" (GRAS) by the US Food and Drug Administration (FDA). Furthermore, the same authors have observed that it is adsorbed on hair, potentially encouraging the permeation of compounds through the skin [13, 14].

It is widely known that the permeation of a compound (both *in vitro* and *in vivo*) can be dramatically affected by the additional presence of other compounds, known as permeation enhancers [15] or retarders [16], including groups of molecules such as surfactants [17]. Surfactants are compounds with surface-active properties and are known to affect the rate and extent of permeation. For this reason, surfactants are widely used in topical formulations [18]. Although they have been shown to enhance permeation under certain circumstances, many studies have demonstrated that their topical use, in cosmetic or pharmaceutical products, can be a problematic issue for the patient, sometimes causing unwanted side effects such as contact dermatitis and irritation [19]. For this reason new detergents, such as biosurfactants, that avoid these issues, are currently highly desirable as one potential solution for drug delivery [20], although their effect on skin permeation is yet unknown.

Skin is the largest organ in the human body, generally seen as a useful barrier against the unwanted transfer of undesirable contaminants. However, dermatological pharmaceutical formulations, such as foams, creams and gels, can take advantage of the skins permeation potential for systemic delivery, often chosen because of their non-invasive nature and ease of application for the patient with anticipated growth in the market [21]. In order to study the permeation behaviour of compounds and formulations considered for transdermal delivery, human skin models are viewed as the best surrogate for clinical data [22, 23], yet present many ethical, economic and technical problems concerning availability [24]. For example, skin samples require complex storage, suffer from reproducibility as a consequence of variations in skin absorption across different body sites and can be costly studies to undertake. For this reason the majority of transdermal analysis is undertaken using animal skin, such as pig, mouse, rat, guinea pig or even snake skin [25]. However, animal tissues also have several disadvantages and challenges, with the most significant issue being the frequent lack of correlation between human and animal skin data [26]. In recent years, several *in vitro* techniques have been developed to determine topical permeation data, thus avoiding (incomparable) animal or (complex) human models [27]. One such group of alternatives is based on the use of polymer materials [28], including polydimethoxysiloxane (PDMS), as a barrier membrane for permeation that can be used in diffusion systems. PDMS represents a simple alternative option (in comparison with the systems previously discussed) overcoming many of their disadvantages such as cost, complexity and ethical considerations. Moreover, PDMS is particularly useful for studying basic permeation mechanisms, and ranking drugs and formulations through comparison of their permeation profiles [16, 29, 30]. For example, PDMS membrane has been reported to produce data displaying a good correlation with an *in vivo* system whereby the penetrant lipophilicity was the prime determinant of compound permeation [31].

Therefore, the aim of this work was to evaluate the effect of a biosurfactant extract obtained from a corn wet-milling industry stream, on the permeation of a set of model drugs, through a silicone membrane-based diffusion system. Ten compounds were selected for analysis that covered a wide range of physicochemical properties such as variations in hydrophobicity (reflected in logP values), degrees of ionisation (reflected in pKa values), molecular mass and polar surface area, along with the relative acidity/basicity of the compound. This resulted in a set of model compounds that would ultimately facilitate the analysis of a relationship between the degree of permeation and the compounds properties driving the process. The ten

compounds analysed in this work were: benzocaine, benzoic acid, benzotriazole, caffeine, ibuprofen, indomethacin, lidocaine, procaine, salicylic acid and tetracaine.

Overall, in this study, the permeation of ten compounds through PDMS was measured in the presence and absence of two concentrations of an extracted biosurfactant, i.e. above and below its critical micellar concentration (CMC), in order to establish the effect of biosurfactant presence and micelle formation in permeation studies.

Materials and Methods

Materials

Polydimethylsiloxane membrane (PDMS) was used as purchased (ATOS Medical, Sweden) with a standard thickness of 130 µm and cut to size as required. Ten pharmaceutical compounds were analysed in this study namely: benzocaine (Sigma Aldrich), benzoic acid (Sigma Aldrich), benzotriazole (Sigma Aldrich), caffeine (Sigma Aldrich), ibuprofen (BASF), indomethacin (Sigma Aldrich), lidocaine (Sigma Aldrich), procaine (Sigma Aldrich), salicylic acid (Sigma Aldrich) and tetracaine (Sigma Aldrich), all with a minimum purity of 99 %. Buffer (0.02 M, pH 7.4) consisted of dipotassium hydrogen phosphate (> 98 %, Fisher Scientific) and monopotassium hydrogen phosphate (> 99 %, Fisher Scientific) with 0.1 M sodium chloride (> 99 % Sigma Aldrich).

The biosurfactant extract was obtained from corn steep liquor (CSL) provided by Santa Cruz Biotechnology (Lot L1813), using chloroform stabilised with amilene (150 ppm) as organic solvent (Scharlab).

Methods

Biosurfactant extraction from corn steep liquor

For obtaining the biosurfactant, CSL with a solid content of 50 % was subjected to an extraction process following the protocol established by Vecino *et al*., [32]. For that, CSL was diluted in distilled water up to 50 mg/mL. Then, 250 mL of this solution was extracted with chloroform at 56 ºC for 1 hour with a CSL:chloroform ratio 2:1 (v/v), in a KS 4000 ic control shaker (IKA, Germany). Finally, the chloroform-CSL mixture was decanted for 12 hours. Once the biosurfactant was extracted, the organic solvent was eliminated by vacuum distillation obtaining an oily biosurfactant extract.

Physical characterisation of biosurfactant extract

The surfactant activity of extract from CSL was measured using the Wilhelmy plate method and a tensiometer K20 (Kruss). For that, the biosurfactant was dissolved in water at concentrations from 0.01 to 1 mg/mL (the concentrations prepared were 0.01; 002; 0.05; 0.1;

0.125; 0.166; 0.2; 0.25; 0.5 and 1 mg/mL), in order to obtain its critical micellar concentration (CMC), with all measurements carried out in triplicate.

The pH of biosurfactant was measured using a calibrated pH meter Basic 20 (Crison) and its ionic charge was determined using ion exchange resins following the established protocol [33]. The resins used for studying the ionic charge of the bisourfactant were IR 120 (cationic exchange resin) and IRA 400 (anionic exchange resin). For these measurements 10 mL of biosurfactant was dissolved at concentrations of 1 mg/mL in distilled water and were placed in contact with 1 g of resins, observing changes in the surface tension of water after 30 min. As surface tension is directly related to the amount of biosurfactant entrapped by the resin, this parameter allowed determination of the presence (or absence) of biosurfactant in the remaining solution.

Chemical characterisation of biosurfactant extract

The fatty acid composition of the biosurfactant was analysed using gas chromatography coupled to a mass spectrometer (CG-MS, Bruker Scion 451-GC) and following ISO-12966-3:2009. 10 mg of sample was diluted in 500 µL of tert-butyl methyl ether (TBME), and 250 µL of trimethylsulfonium hydroxide (TMSH). A volume of 1 µL of the sample was injected in split less mode, and the separation of fatty acid methyl esters (FAMEs) was carried out. The column used was a DB-WAX (30 m x 0.25 mm i.d. x 0.25 µL film thickness), heated to 250 ºC at a rate of 4 ºC/min. The carrier gas was helium, with a flow rate of 1 mL/min. A temperature of 250 ºC was set for the injector inlet and the transfer line of the detector.

Finally, a mass selective detector was used for mass spectra acquisition, over an m/z range of 50-400, and under electron impact ionisation at a voltage of 70 eV. FAMEs were identified by comparison of retention time and mass spectra of a FAME standard mix (Supelco 37 Component FAME Mix: 10 mg mL of the FAME reference standard mix in methylene chloride, Sigma-Aldrich). This standard mix includes the fatty acid methyl esters reflected in Table 1**.** The software employed was MS Data Review (Version 8.1).

Table 1: Composition of Supelco 37 FAME Standard Mix.

Compound	Concentration (wt %)
cis-13,16.docosadienoic acid methyl ester	2
cis-4,7,10,13,16,19-docosahexaenoic acid methyl ester	2
cis-11,14-Eicosadienoic acid methyl ester	2
cis-5,8,11,14,17-Eicosapentaenoic acid methyl ester	2

cis-8,11,14-Eicosatrienoic acid methyl ester	2
cis-11,14,17-Eicosatrienoic acid methyl ester	2
cis-11-Eicosenoic acid methyl ester	2
Methyl *cis*-10-heptadecenoate	2
Methyl hexanoate	4
Methyl γ-linolenate	2
Methyl arachidate	4
Methyl arachidonate	2
Methyl behenate	4
Methyl behenate	4
Methyl decanoate	4
Methyl dodecanoate	4
Methyl elaidate	2
Methyl erucate	2
Methyl heneicosanoate	2
Methyl heptadecanoate	2
Methyl linoleate	2
Methyl linolelaidate	2
Methyl linolenate	2
Methyl myristate	4
Methyl myristoleate	2
Methyl oleate	4
Methyl octanoate	4
Methyl palmitate	6
Methyl palmitoleate	2
Methyl pentadecanoate	2
Methyl *cis*-10-pentadecenoate	2
Methyl stearate	4
Methyl tricosanoate	2
Methyl tetracosanoate	4
Methyl tridecanoate	2
Methyl undecanoate	2

Methyl *cis*-15-tetracosenoate	2

The functional groups of the biosurfactant were analysed by Fourier-transform infrared spectroscopy (FTIR). For that, pellets containing 1 mg of biosurfactant and 10 mg of potassium bromide were prepared. Infrared absorption analysis was carried out with a Niocolet 6700 FTIR system (Thermo Scientific) from 400 to 4000 cm^{-1}, with a spectral resolution of 4 cm^{-1}.

Permeation experiments

PDMS membrane was soaked in phosphate buffer solution for 30 minutes prior to being mounted in the flow-through diffusion cells (PermeGear Inc., USA). After assembly the cells were placed on a cell warmer, maintained at 32 °C. 0.8 mL of the donor solution containing model compound and biosurfactant (except in the control, which contained only model compound) was added to the cell. In all experiments, saturated drug solutions were prepared whereby the resultant concentration of the model compounds in the donor solution was between 1.2 and 31.9 mg/mL with biosurfactant extract present at concentrations of 0.005 mg/mL or 0.500 mg/mL. Phosphate buffer saline was pumped through the cells at ≤ 5.0 mL/h. Samples were collected by means of a fraction collector every 45 minutes for a total of 6 hours. Quantification was undertaken using UV spectroscopy (Agilent Cary 60 fitted with a Cary single cell Peltier) with the sample compartment maintained at 35 ºC at the determined λ_{max} for each compound. All experiments were conducted in triplicate with the mean value shown and standard deviation based error limits. All flow-through cells used in this study had a diffusion area of 0.554 cm^2.

Results & Discussion

Characterisation of biosurfactant extract

The industrial applications of biosurfactants will depend on their properties, including their composition, their ionic charge, CMC, and ability to form micelles at low concentrations. For example, those biosurfactants with lower CMCs will produce micelles at a comparatively low concentration which is favourable from an industrial point of view. The biosurfactant under evaluation was found to have a CMC of 0.1198 mg/mL, which is very low in comparison with other biosurfactants reported in literature. For instance, researchers have reported that a biosurfactant produced by *Lactococcus lactis* exhibits a CMC of 14 mg/mL [34], whereas Madhu and Prapulla [35] reported that a biosurfactant produced by *Lactobacillus plantarum*

has a CMC of 6 mg/mL. Biosurfactants produced by lactic acid bacteria are of particular interest for comparative analysis with the biosurfactant under evaluation in this work because lactic acid bacteria are also known to be involved during the steeping process of corn.

Another important characteristic of biosurfactants is their ionic charge. Surfactants are amphipathic molecules consisting of hydrophobic (oil soluble) and hydrophilic (water soluble) moieties in one molecule. According to their charge, surfactants can be classified as anionic, cationic, non-ionic or amphoteric [36]. The biosurfactant under evaluation in this work was found to be amphoteric as it was entrapped by anionic and cationic resins. More specifically, IRA400 was able to entrap 75 % of the biosurfactant present in the solution, whereas IR120 entrapped 62 % of biosurfactant. These results demonstrate the amphoteric nature of this biosurfactant, which is in concordance with data previously reported [33]. Amphoteric surfactants are ideal excipients, widely used in personal care products, as they tend to be less irritating to skin and eyes, have high biological compatibility and low toxicity in comparison with other types of surfactants [37]. A summary of the properties of the biosurfactant extracted for this study is shown in Table 2.

Table 2: Physical properties of the biosurfactant extract used in this work.

Critical Micellar Concentration (mg/mL)	0.1198 (± 0.026)
Minimum Surface Tension (mN/m)	41.8 (± 0.4)
pH	4.5
Adsorption on IRA (400)	positive
Adsorption on IR 120	positive

Related to the fatty acid composition, FAME analysis confirmed that fatty acids represented almost 43 % of the biosurfactant extract. These fatty acids contained 16 and 18 C units, such as palmitic, stearic, oleic, and linolelaidic acids, which represented 2 %, 7 %, 12 % and 22 % of the biosurfactant extract, respectively. These results confirm that this extract has a significant lipophilic character, as seen previously [33, 38]. As a result of the lipidic nature of this biosurfactant, it is proposed that it could improve the permeation of drugs through the skin. This theory is based upon the knowledge that the stratum corneum of skin is composed of lipidic compounds such as cholesterol and similar fatty acids to those found in this biosurfactant extract [39].

FTIR analysis of the biosurfactant extract (Supplementary Figure S1) displayed similar absorption bands to those obtained in previous work, demonstrating that the production of

this bisourfactant extract in corn steep liquor is regular and homogeneous [40]. The FTIR spectrum presented strong absorption bands between 3000 cm^{-1} and 2700 cm^{-1}, indicating the presence of carboxylic acids present in fatty acid esters and proteins, along with an absorption band at ~1700 cm^{-1} indicative of C=O stretch. Absorption bands between 1500 cm^{-1} and 1300 cm^{-1} are indicative of symmetric and asymmetric N-O stretch, present in amino acids.

Permeation properties

It should firstly be noted that as the amount permeated varied (depending upon solubility in the modified buffer solution), all comparative analysis was undertaken by comparing the percentage change in amount rather than the mass permeated per cm^2. For five of the ten compounds analysed, the presence of the biosurfactant significantly increased the cumulative amount of compound permeated after six hours, compared with drug alone. The increase in cumulative permeation did appear to vary depending upon the compound under investigation, ranging from a maximum increase for benzoic acid of 47 % (Figure 1) through to only a 15 % maximum increase for salicylic acid. The increase in amount permeated also appeared to be dependent upon the amount of biosurfactant present, as the addition of 0.500 mg/mL had a more profound effect on permeation compared with the addition of 0.005 mg/mL biosurfactant. For lidocaine, the presence of 0.005 mg/mL biosurfactant had no effect on permeation, and interestingly, for salicylic acid the lower biosurfactant concentration increased permeation to a greater extent than that for the higher concentration solution. This could be explained by changes produced in the micelle size, due to an increase in the number of aggregated biosurfactant molecules. For example, Sanchez et al., [41] have reported the formation of larger aggregates at concentrations above the CMC for dirhamnolipid produced by *Peudomonas aeruginosa*. Despite the fact that the maximum concentration of biosurfactant employed was more than 5 times lower than the CMC, Malcher and Gzyl-Malcher [42] have also observed that salicylate ions facilitate the formation of polymer-micelle aggregates by screening the electrostatic repulsions between the charge surfactant head groups. Thus, in this case, salicylic acid contributed to the formation of aggregates

through a synergistic effect to a much greater extent than other compounds.

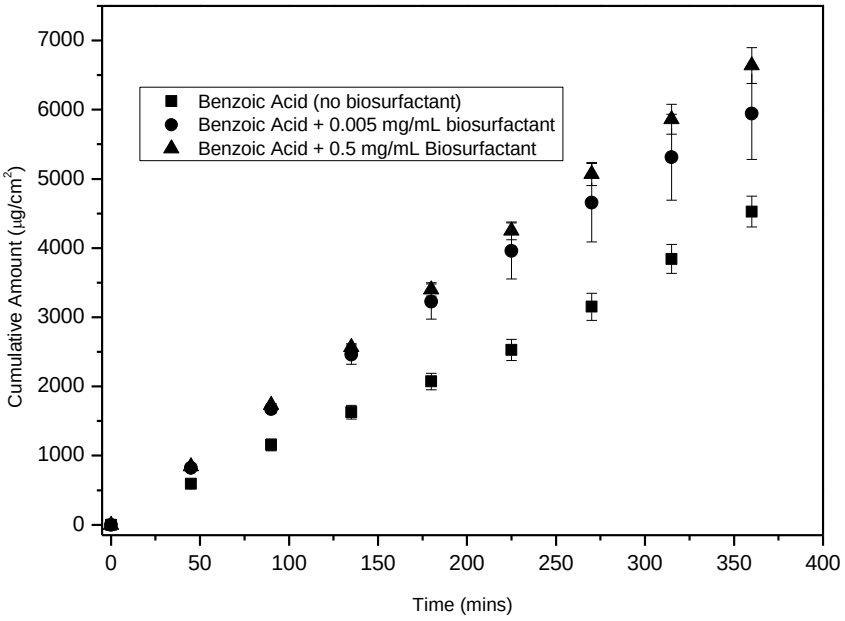


Figure 1: Cumulative amount permeated over six hours for benzoic acid in buffer (■), in the presence of 0.005 mg/mL surfactant (●) and 0.500 mg/mL surfactant (▲), n = 3 and ± = SD.

The cumulative amount percentage increases in permeation for the five compounds (where an increase was observed) are summarised in Table 3.

Table 3: Cumulative amount percentage increase after six hours for the five compounds that displayed an increase in permeation in the presence of biosurfactant.

Compound	% increase in cumulative permeation after six hours in the presence of 0.005 mg/mL biosurfactant	% increase in cumulative permeation after six hours in the presence of 0.500 mg/mL biosurfactant
Benzocaine	23 (± 1 %)	37 (± 2 %)
Benzoic Acid	31 (± 5 %)	47 (± 1 %)
Caffeine	17 (± 6 %)	25 (± 7 %)
Lidocaine	-6 (± 1 %)	24 (± 7 %)
Salicylic Acid	43 (± 6 %)	15 (± 4 %)

For one of the ten compounds analysed, namely benzotriazole, the presence of the biosurfactant had no significant effect on the cumulative amount of compound permeated after six hours compared with compound alone (Figure 2). This compound is known to self-

assemble in aqueous solution to form aggregated structures [43] which may have affected the permeation process.

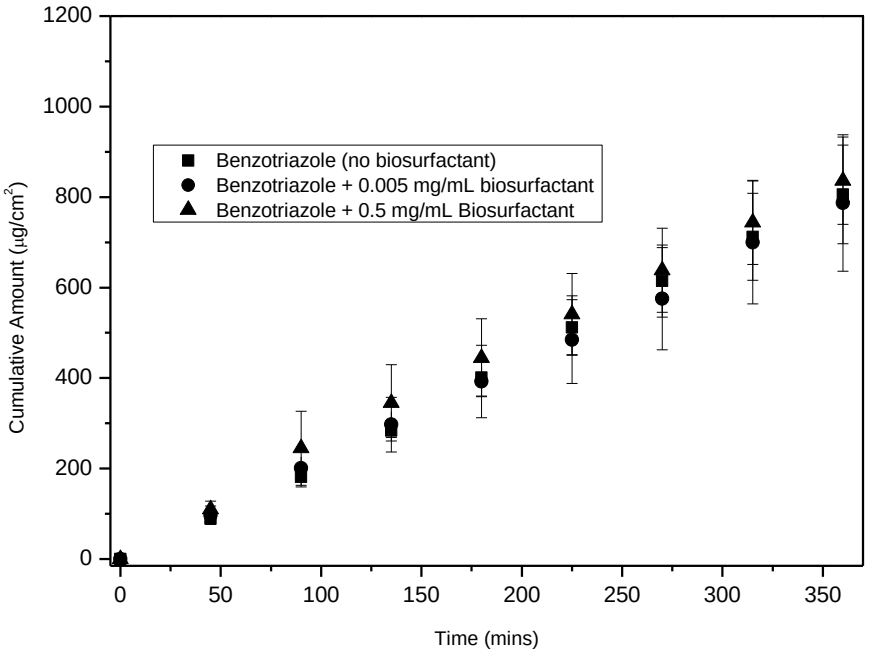
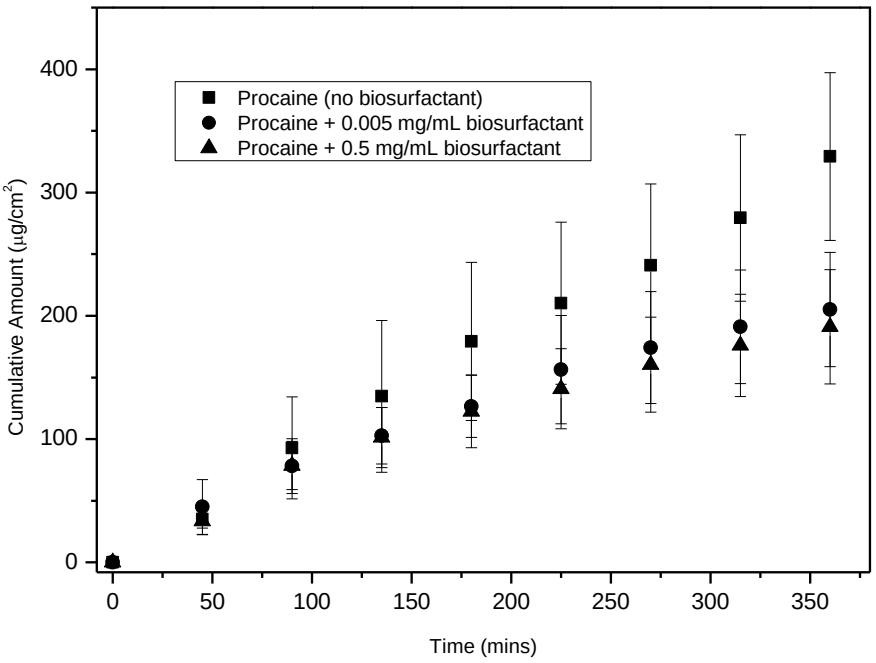


Figure 2: Cumulative amount permeated over six hours for benzotriazole in buffer (■), in the presence of 0.005 mg/mL surfactant (●) and 0.500 mg/mL surfactant (▲), n = 3 and ± = SD.

For the remaining four of the ten compounds analysed the presence of the biosurfactant significantly decreased the cumulative amount of compound permeated after six hours compared with compound alone. The decrease in cumulative permeation once again, did appear to vary depending upon the compound under investigation ranging from a maximum decrease for procaine of 36 % (Figure 3), through to only a 13 % maximum decrease for ibuprofen. Furthermore, the decrease in amount permeated appeared to be less dependent upon the amount of surfactant present (compared with the compounds where an increase in permeation was observed), whereby the addition of 0.500 mg/mL only decreased permeation for one of the compounds (indomethacin) compared with the addition of 0.005 mg/mL biosurfactant.



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276 Figure 3: Cumulative amount permeated over six hours for procaine in buffer (■), in the
277 presence of 0.005 mg/mL surfactant (●) and 0.500 mg/mL surfactant (▲), n = 3 and ± = SD.

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279 The cumulative amount percentage decreases in permeation for the four compounds (where a
280 decrease was observed) are summarised in Table 4.

281

282 Table 4: Cumulative amount percentage decrease after six hours for the four compounds that
283 displayed a decrease in permeation in the presence of biosurfactant.

Compound	% decrease in cumulative permeation after six hours in the presence of 0.005 mg/mL biosurfactant	% decrease in cumulative permeation after six hours in the presence of 0.500 mg/mL biosurfactant
Ibuprofen	23 (± 2 %)	13 (± 1%)
Indomethacin	21 (± 6 %)	28 (± 3 %)
Procaine	42 (± 1 %)	36 (± 3 %)
Tetracaine	17 (± 5 %)	16 (± 4 %)

284

To explain why the ten compounds exhibited such variable effects in the presence of biosurfactant several physicochemical properties relating to the compounds were considered, including lipophilicity, degree of ionisation at the experimental pH, pKa, solubility, polar surface area and molecular mass. For example, it is interesting to note that procaine has a similar chemical structure to lidocaine with the minor difference being an amine and ester group on the former and an amide on the latter. This does result in procaine being less lipophilic than lidocaine and assuming the two drugs each bind to the micelle through electrostatic attractions, it may be that procaine has a greater preference to remain in the aqueous phase, rather than being incorporated within micelles and is therefore not encouraged to permeate. However, lipophilicity was deemed not to be a determining factor as to whether a compound experienced an increase or decrease in permeation. For example, a similar decrease in permeation was observed for indomethacin and procaine at the higher biosurfactant concentration, yet the two compounds have significantly different lipophilicities (calculated using Advanced Chemistry Development, Inc. (ACD/Labs) to be 4.3 and 3.1 respectively).

After analysing all of these factors it was found that the primary factor dictating permeation was the relative molecular mass of the compound. For the five compounds that experienced an increase in permeation in the presence of surfactant, the relative molecular mass was below 200 whereas for the four that experienced a decrease in permeation the relative molecular mass was above 200. However, there was one exception to this correlation, namely lidocaine, with a relative molecular mass of 234 and a maximum increase in permeation of 24 %. This anomaly may be a consequence of the amphiphilic nature of the drug allowing it to interact to a greater extent than anticipated with the biosurfactant. It is theorised that in general, a strong interaction was possible between a compound and the biosurfactant if the compound was relatively small (as indicated by a comparatively small relative molecular mass) and it is this interaction that helps encourage permeation to occur. In contrast, for the four compounds with comparatively high molecular masses, i.e. > 200, the compound-biosurfactant interaction either did not occur or resulted in a large, complex structure that struggled to permeate the silicone membrane. This theory fits well with that previously published in literature where the correlation between reduced diffusion with increasing penetrant size is well documented [44]. Overall, the findings presented in this work indicate that there is potential for the application of biosurfactants within formulations as an aid to modify transdermal permeation and that this effect can be controlled by the choice of compound selected.

Conclusion

In summary, the cumulative amount of permeation for ten compounds through silicone membrane in the absence and presence of a naturally occurring biosurfactant was analysed. Overall, the effect on permeation appeared to vary between the compounds, increasing permeation in some cases yet decreasing permeation in others, possibly linked to the compounds relative molecular mass as a reflection of molecular size. It would appear that those with a comparatively small mass are able to permeate more easily and to a greater extent, possibly through favourable compound-biosurfactant interactions, whereas the reverse scenario is true for compounds of a high molecular mass in that permeation is retarded. These findings are of interest for those considering the incorporation of biosurfactants in pharmaceutical, cosmetic or household products. There are many reasons why biosurfactants could be used within formulations, for example their low toxicity, ability to biodegrade and biocompatibility. However, it should also be considered that they can be more complex to obtain in a pure form, with variable composition and expensive to extract on a large scale. Overall though, the advantages offered by the inclusion of biosurfactants are significant and it is anticipated that more and more biosurfactant-based products will appear in future years.

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Supplementary Information

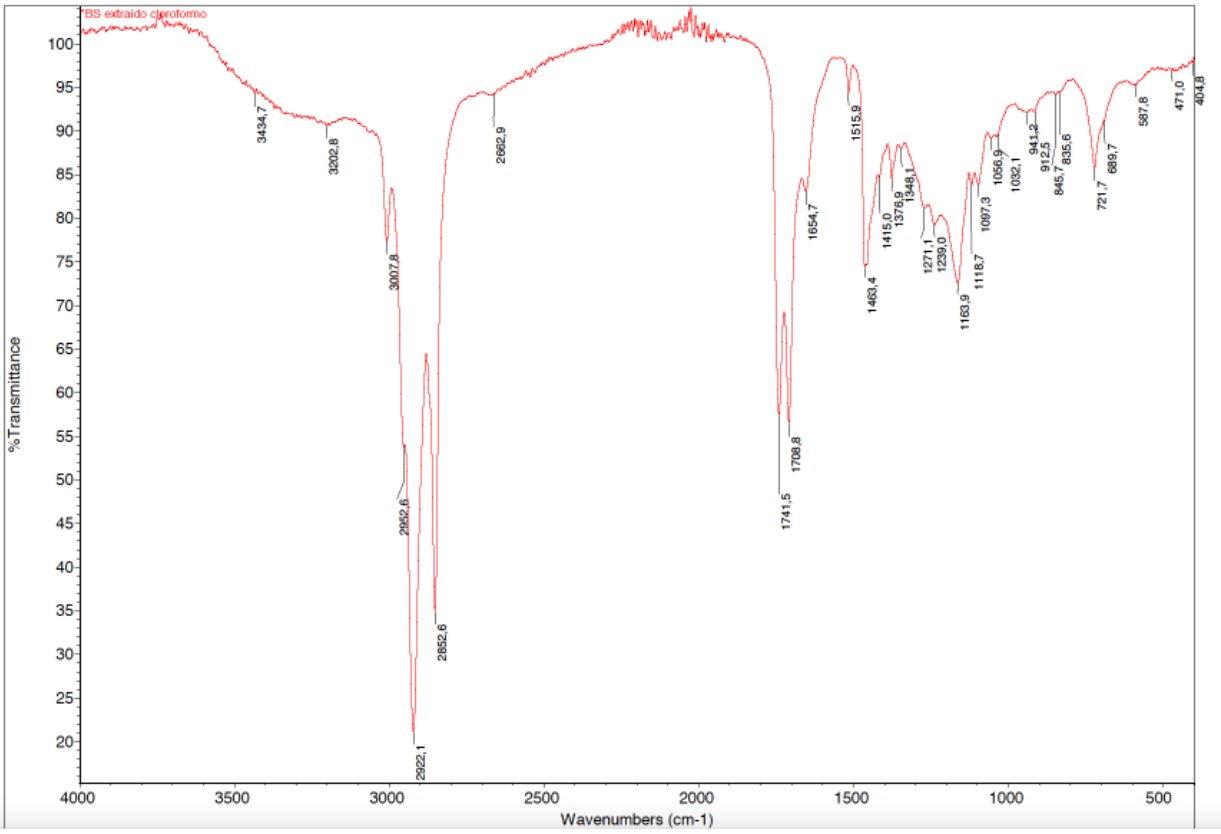

Figure S1: FTIR spectra of biosurfactant extract from CSL.