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The influence of Leucidal – eco-preservative from radish – on model lipid membranes and selected pathogenic bacteria

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ABSTRACT

In this work the effect of Leucidal - a natural preservative from radish dedicated to be used in cosmetics - on bacteria cells and model bacteria membranes was investigated. To get insight into the mechanism of action of this formulation the lipid Langmuir monolayers imitating *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) membranes were prepared. Then, the influence of Leucidal on model systems was investigated by means of the surface pressure/area measurements, penetration studies and Brewster Angle Microscopy (BAM) visualization. Similar experiments were done also for one component monolayers formed from the model membrane lipids. The in vitro tests were done on five different bacteria species (*E. coli, Enterococcus faecalis, S. aureus, Salmonella enterica, Pseudomonas aeruginosa*). Leucidal was found to decrease packing of the monolayers, however, it was excluded from the films at higher concentrations. Model membrane experiments evidenced also a stronger affinity of the components of this eco-preservative to *E. coli* vs *S. aureus* membrane. Among one component films, those formed from phosphatidylglycerols and cardiolipins were more sensitive to the presence of Leucidal. However, in vitro tests evidenced that Leucidal exerts stronger inhibitory effect against *S. aureus* bacteria as compared to *E. coli* strain. These findings were discussed from the point of view of the role of Leucidal components and the lipid membrane properties in the membrane - based mechanism of action of this preservative. The results allow one to suggest that the membrane may not be the main site of action of Leucidal on bacteria. Moreover, since high concentration of the tested preparation exerted antibacterial activity in relation to all tested bacteria, a low selectivity of Leucidal can be postulated, which may be problematic from the point of view of its effect on the skin microbiome.

1. Introduction

Natural or synthetic substances, which are added to food products, pharmaceuticals or cosmetics to prolong their shelf life, maintain their microbial purity and prevent undesirable chemical changes are called preservatives (Deza and Giménez-Arnau, 2017; Kamala Kumari et al., 2019; Herman, 2019; Halla et al., 2018). Even before preservatives were discovered, people used various techniques to prevent food spoilage, such as drying food, preparing solutions of a high content of sugar (jams, jellies) or salt level (salting meat) (Kamala Kumari et al., 2019). The possibility of microbial contamination is also a serious problem in the cosmetics industry. It can occur at all stages of the production of cosmetics, as well as during their use. According to the norm PN-EN ISO 17516:2014 (PN-EN ISO 17516, 2014), the quality tests cannot identify *E. coli, S. aureus, P. aeruginosa and C. albicans* in 1 g or 1 mL of the cosmetic. Quantitatively, the acceptable microbiological purity standards are defined according to the cosmetic category. For cosmetics for children, intended for the use around the eyes or on mucous membranes (category I), the maximum allowable amount of aerobic mesophilic bacteria is 100 cfu/g (colony forming units per gram). However, in the case of other cosmetics, the result of the tests cannot exceed 1000 cfu/g. Despite many sources of contamination occurring during manufacturing or packing, the most important is the period after the product is opened and it has a contact with non-sterile fingers or applicators. In addition, storage conditions (temperature, humidity) other than those recommended by the manufacturer can also affect the microbiological stability of the product. Microorganisms can not only affect cosmetic color, fragrance and degrade active compounds, but can also lead to serious

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infections in consumers (Kamala Kumari et al., 2019; Kerdudo et al., 2016; Varvaresou et al., 2009). Therefore, the use of preservatives or other preservation techniques is very important and necessary (Kerdudo et al., 2016). Good preservatives must have a broad spectrum of bacteria and fungi against which they can be active, be stable under changing conditions, be compatible with all product ingredients and not affect the physical properties of cosmetics. Furthermore, they should be safe for human health and the environment (Deza and Giménez-Arnau, 2017). Currently, commonly used synthetic preservatives include isothiazolinones, iodopropynyl butylcarbamate, organic acids and parabens. In addition to their broad spectrum of antimicrobial activity and compatibility with other ingredients, their price is also relatively low (Deza and Giménez-Arnau, 2017; Herman, 2019; Kerdudo et al., 2016).

Nowadays, many preservatives are associated negatively, which originates from the fact that they can be a common factor of allergic reactions and skin, eye and lung irritation (Deza and Giménez-Arnau, 2017; Kamala Kumari et al., 2019). In this context, special attention should be paid to parabens and the potential risks associated with their use. They are esters of 4-hydroxybenzoic acid, which have estrogen mimicking properties that might be linked with increasing breast cancer occurrence. A high levels of parabens may also influence the body homeostasis, reduce a quality of semen and be one of the factors causing obesity and immunological disorders (Kerdudo et al., 2016; Nowak et al., 2021). When a woman is pregnant, they also affect fetal development, especially the brain growth (Kamala Kumari et al., 2019). In European Union the rules in cosmetic industry are strictly regulated by the Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products (REGULATION EC, 2022). In Annex V of this regulation the preservatives allowed in the cosmetic products are listed. Remaining annexes include the lists of colorants or UV filters allowed in cosmetics, the prohibited substances and a list of compounds that cosmetic products must not contain, unless they are subject to established restrictions (REGULATION EC, 2022). However, there is a systematic search for new preservatives, for various applications. In the cosmetic industry, the interest is focused on plant extracts and various substances of plant origin. Many plant derivatives such as tea (*Camellia sinensis*) extract have a positive influence on the condition of our skin or hair condition. They are currently used in cosmetic products and generally, they do not cause skin irritation or allergies. They are classified as Generally Recognized as Safe and they are considered to be safer for human health and the environment as compared to synthetic substances (Nowak et al., 2021; Simões et al., 2009).

Additionally, many plants and substances of natural origin have proven antimicrobial activity. Many of them show antimicrobial effect in cosmetic formulations, such as *Thymus vulgaris* essential oil having activity against *S. aureus*, *P. aeruginosa* and *E. coli* in particular types of formulations (Herman, 2019; Bello et al., 2022; Alvarez-Rivera et al., 2018). All this has led to a trend of replacing synthetic preservatives in cosmetics with natural antimicrobial substances (Nowak et al., 2021).

In addition to classifying preservatives into natural and synthetic, these substances can be divided into many groups according to their mechanism of action and chemical structure. For example, organic acids act on microorganisms by acidifying the external environment or cytoplasm, or by penetrating uncharged organic acids into cells, where their charge changes to negative and they affect the internal pH value. Alcohols act on microorganisms by denaturing proteins or inhibiting protein synthesis (Halla et al., 2018; Alvarez-Rivera et al., 2018).

The aim of our studies was to verify the antibacterial effect of a commercially available natural preservative obtained from radish - Leucidal® Liquid (Leucidal) on selected species of pathogenic bacteria and to investigate the influence of this formulation on model lipid bacterial membranes.

According to the information provided by the producer, Leucidal® Liquid contains novel antimicrobial peptide (AMP), the antimicrobial activity of which was confirmed in the tests (Technical Dossier Leucidal® Liquid, 2022). Leucidal® Liquid is a water-based formulation of pH = 4–6; the formulation contains also salicylic acid (18–22%) and it is active both at acidic (pH 3) and basic conditions (pH 8) (Technical Dossier Leucidal® Liquid, 2022). Additionally, as reported in literature (Herman, 2019; Li et al., 2015) the analysis of commercial Leuconostoc/radish root ferment filtrates evidenced that in the studied formulation a salicylic acid is the component active against Gram-negative bacteria, while didecyldimethylammonium salt is responsible for the activity against Gram-positive species (Li et al., 2015). In addition, during the analysis it was unable to detect antimicrobial peptides in the studied samples of fermented radish root filtrate (Li et al., 2015).

Our intention was to test the antimicrobial activity of this preservative and to gain insight into its mechanism of action. The latter issue is crucial from the point of view of the practical application of any biologically active substance (e.g. the possibility of expanding its use or enhancing its effect). One of the targets of bioactive compounds in cells is the membrane. It is clear that the composition of the cellular membrane is very complex. It is formed by many types of lipids, arranged in a bilayer with many types of proteins embedded, and the composition of this structure determines its properties (Yeagle, 1989). Therefore, for investigating the influence of a particular substance on a membrane, the application of one of the popular membrane models seems to be a good choice. The most popular membrane models widely used in the investigations of membrane-active substances are the lipid Langmuir monolayers and liposomes (Stefaniu et al., 2014; Akbarzadeh et al., 2013; Hąc-Wydro and Dynarowicz-Łątka, 2010). Thus, in our studies, the influence of a commercially available product, Leucidal® Liquid, on the mixed lipid monolayers mimicking the membranes of a Gram-positive and Gram-negative bacteria (*S. aureus* and *E. coli*, respectively) was investigated. Moreover, to get some insight into the role of individual lipids in the mechanism of action of the studied preservative the experiments on the effect of Leucidal on one component lipid films were also performed. Based on the results of our in vitro test and model membrane experiments the effect of Leucidal on lipid membranes was discussed in the context of the possible mechanism of action of this preservative.

2. Materials and methods

2.1. Antibacterial activity experiments

The studies on antibacterial activity of Leucidal (Leucidal® Liquid was purchased from online cosmetic shop escent.pl (Online cosmetic shop esent.pl, 2022)) were performed against selected pathogenic bacteria, which are among the most common causes of various infections in humans. The strains were purchased from the American Type Culture Collection (ATCC): *Staphylococcus aureus* ATCC 29213, *Salmonella enterica* ATCC BAA664, *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 15922, and *Enterococcus faecalis* ATCC 29212, stored frozen at minus 72°C and revived immediately prior to testing. Antimicrobial activity was investigated using a modified Hancock Lab microdilution procedure (Modified MIC Method for Cationic Antimicrobial Peptides. Available online: (Modified MIC, 2023)) in flat-bottom, polystyrene, 96-well microdilution plates (Nest, China).

Bacterial inocula were prepared from 24-hours cultures on Tryptic Soy Agar (Becton-Dickinson) by suspending a few colonies in sterile distilled water to obtain 0.5 McFarland cell density and later diluted 200-fold in Miller Hinton Broth II (MHB) (Merk, Germany). From Leucidal preparation, a series of dilutions in water was prepared giving intermediate concentrations ranging from 10 μg/mL to 0.54 g/mL.

Microdilution plates were filled with 20 μL of the corresponding concentration of examined products and inoculated with 180 μL of bacterial suspension in MHB medium. The final bacterial inocula concentrations on the microtiter plates were approx. 1–2.5 × 10^5 CFU/mL and the concentrations of tested substance were: 1 μg/mL, 5 μg/mL, 10 μg/mL, 50 μg/mL, 0.1 mg/mL, 0.5 mg/mL, 1 mg/mL, 5 mg/mL, 10 mg/

mL, 50 mg/mL. The tests were performed in triplicate.

The plates were incubated without agitation at 35 ± 2°C in ambient air for 24 h. Afterwards, automatic readings were performed with a microdilution plate reader (Tecan, Sunrise) and the absorbance at 530 nm wavelength was measured. The evaluation of antimicrobial properties was based on the percentage comparison of the growth of microorganisms in the presence of Leucidal extract to the bacterial growth without the preservative in the same conditions. The minimal inhibitory concentration (MIC) of Leucidal product was considered to be the lowest concentration causing at least 95% inhibition of bacterial growth compared to the growth control without the addition of the test substance.

After automatic readings of the microplates the contents of the wells were transferred on Tryptic Soy Agar (Becton-Dickinson) to determine the minimal bactericidal concentration (MBC) of Leucidal product. The inoculated media were incubated at 35 ± 2°C in ambient air for 24 h and after assessed visually for the presence of bacterial colonies. MBC was defined as the lowest concentration of preservative for which no bacterial growth was observed on the medium.

2.2. Monolayer experiments

2.2.1. Materials

Synthetic lipids: 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (POPE), 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-(1′-rac-glycerol) (sodium salt) (POPG), 1′,3′-bis[1,2-dioleoyl-sn-glycero-3-phospho]-glycerol (sodium salt) (TOCL), 1′,3′-bis[1,2-distearoyl-sn-glycero-3-phospho]-glycerol (sodium salt) (TSCL) and 1,2-dipentadecanoyl-sn-glycero-3-phospho-(1′-rac-glycerol) (sodium salt) (PG 15:0) were the compounds of high purity (≥99%) purchased from Avanti Polar Lipids Inc., USA. Lipids were dissolved in chloroform/methanol (9:1 v/v) mixture (HPLC grade, ≥99.9%, Aldrich). Model membranes were composed of major lipid classes characteristic for particular bacterial species. *E. coli* model membrane consists of POPE, POPG and TOCL (75%, 20%, 5%, respectively, by mole %). *S. aureus* model membrane consists of PG15:0 and TSCL (58% and 42%, respectively, by mole %) (Epand and Epand, 2009). The salts for preparation of phosphate buffer saline (PBS): sodium chloride, potassium chloride, disodium hydrogen phosphate and sodium dihydrogen phosphate of a purity > 99% were purchased from Avantor Performance Materials Poland S.A. To prepare PBS and in all experiments ultrapure Milli-Q water of conductivity 0.055 μS/cm was used.

2.2.2. Methods

2.2.2.1. The surface pressure (π) – area (A) measurements. The surface pressure (π) – area (A) isotherms for one-component lipid films and for the mixed monolayers mimicking bacterial membranes were recorded. The lipid ratio of the lipids in models was defined above. Monolayers were spread on a buffer and on Leucidal solutions of 1 μg/mL, 5 μg/mL, 10 μg/mL, 25 μg/mL and 50 μg/mL. Leucidal solutions were prepared by dilution of the stock solution (Leucidal® Liquid 50% water solution) in PBS (pH 7.4). PBS was prepared by dissolving particular salts in ultrapure Mili-Q water. The concentration of salts in PBS was as follows NaCl 13.7 mM, KCL 0.27 mM, Na_2HPO_3 0.43 mM and NaH_2PO_3 0.15 mM. The ionic strength of the buffer was 15.0 mM. To form monolayers on the subphase Hamilton microsyringe (± 1.0 μL) was used. Before the compression was started the monolayers were left for 5 min. To preform measurements the KSV-NIMA Langmuir trough (total area=275 cm^2) with two Delrin barriers enabling symmetrical compression of the monolayers was used. The trough was placed on an anti-vibration table. During compression barriers speed was set on 10 cm^2/min and the surface pressure was measured (± 0.1 mN/m) with the Wilhelmy plate made of filter paper (ashless Whatman Chr1) connected to an electrobalance. The temperature value (at 20 °C) was controlled thermostatically (± 0.1 °C) by a circulating water system (Julabo, Germany). All the measurements were repeated at least twice to obtain consistent results (the error for the area per molecule does not exceed 0.2 A^2/molecule).

2.2.2.2. Penetration experiments. To investigate the ability of the components of Leucidal to incorporate into the monolayers the penetration experiments were performed. Measurements were done for both one-component monolayers and the mixed films (model membranes). The monolayer was formed on a PBS solution and compressed up to the target surface pressure. Then, it was left for equilibration to obtain desirable initial surface pressure π_i ($\pi_i = 10$ or 30 mN/m). Then, Leucidal solution was injected into the subphase through the monolayer and the changes of the surface pressure were monitored for ca. 1 h. During experiments, the subphase was continuously stirred. For the mixed monolayers the measurements for $\pi_i = 10$ and 30 mN/m and Leucidal concentration of 5 μg/mL; 10 μg/mL and 50 μg/mL were performed. To estimate the maximum insertion pressure (MIP) and verify the synergy parameter additionally the experiments at 20 mN/m were performed at one Leucidal concetration (50 μg/mL). For one-component lipid monolayer penetration studies for $\pi_i = 10$ and 30 mN/m at the highest investigated Leucidal concentration were done. The results of these experiments were reproducible within 2%.

2.2.2.3. Brewster angle microscopy studies. Morphological changes within monolayers occurring during compression were recorded in Brewster Angle Microscopy (BAM) experiments. Measurements were performed on a buffer subphase and on the highest Leucidal concentration (50 μg/mL). In these experiments, an UltraBAM instrument (Accurion GmbH, Goettingen, Germany) equipped with a 50-mW laser emitting p-polarized light at a wavelength of 658 nm, a 10 × magnification objective, polarizer, analyzer and a CCD camera was used. The spatial resolution of the microscope was 2 μm. The Langmuir trough and Brewster Angle Microscope were placed on a table (Standa Ltd., Vilnius, Lithuania) equipped with an active vibration isolation system (anti-vibration system VarioBasic 40, Halcyonics, Göttingen, Germany).

3. Results

3.1. Leucidal antibacterial activity testing

The results of antibacterial tests are presented in Table 1, Table 2 and in Fig. 1. MIC (minimal inhibitory concentration) values for Leucidal against studied bacterial species were 50 mg/mL, except *S. enterica* for which the concentration 10 mg/mL caused ≥ 95% growth inhibition. MBC (minimal bactericidal concentration) value was determined only against *P. aeruginosa* and the highest tested Leucidal concentration (50 mg/mL). For the remaining species MBC could not be established in the concentration range used in the study.

Based on these results the foregoing conclusions can be drawn. Bactericidal effect of Leucidal was demonstrated only against *P. aeruginosa*, however, only for the highest tested concentration (50 mg/mL). Moreover, for this species the concentrations below 50 mg/mL caused very slight inhibition of bacterial growth. For the other tested species, only bacteriostatic activity was observed and the degree of growth inhibition compared to growth control could be read. Namely, at low concentrations (up to 1 mg/mL) Leucidal demonstrates the best inhibitory effect against Gram-positive *S. aureus* bacteria. In the concentration range from 1 μg/mL to 1 mg/mL the antibacterial effect of preservative decreases in the order: *S. aureus*>*E. coli*>*S. enterica*>*P. aeruginosa* and *E. faecalis*, which corresponds to the degree of inhibition of bacterial growth in a given concentration of the tested preparation (Table 1). The increase of Leucidal concentrations (from 5 to 50 mg/mL) slightly changes the foregoing order. Namely, the strongest effect appears for *S. enterica*, then for *S. aureus* and *E. coli*. Comparing two species whose cell membrane models were used in

Table 1
Antibacterial activity of Leucidal.

The percentage of bacterial growth in the presence of tested substance in relation to the control growth without the addition of the substance					
Leucidal concentration	*Escherichia coli* ATCC 25922	*Enterococcus faecalis* ATCC 29212	*Staphylococcus aureus* ATCC 29213	*Salmonella enterica* BAA-664	*Pseudomonas aeruginosa* ATCC 9027
1 μg/mL	≥ 95%	93%	80%	≥ 95%	≥ 95%
5 μg/mL	≥ 95%	≥ 95%	88%	≥ 95%	≥ 95%
10 μg/mL	≥ 95%	≥ 95%	90%	≥ 95%	≥ 95%
50 μg/mL	≥ 95%	≥ 95%	83%	≥ 95%	≥ 95%
0.1 mg/mL	≥ 95%	≥ 95%	82%	≥ 95%	≥ 95%
0.5 mg/mL	87%	≥ 95%	73%	91%	≥ 95%
1 mg/mL	80%	≥ 95%	53%	84%	≥ 95%
5 mg/mL	46%	≥ 95%	33%	28%	≥ 95%
10 mg/mL	17%	≥ 95%	13%	≤ 5%	87%
50 mg/mL	≤ 5%	≤ 5%	≤ 5%	≤ 5%	≤ 5%

Table 2
Leucidal minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) against tested bacteria.

Bacterial species	MIC	MBC
Escherichia coli ATCC 25922	50 mg/mL	not established*
Enterococcus faecalis ATCC 29212	50 mg/mL	not established*
Staphylococcus aureus ATCC 29213	50 mg/mL	not established*
Salmonella enterica BAA-664	10 mg/mL	not established*
Pseudomonas aeruginosa ATCC 9027	50 mg/mL	50 mg/mL

* Leucidal extract in the tested concentration range did not exhibit bactericidal activity

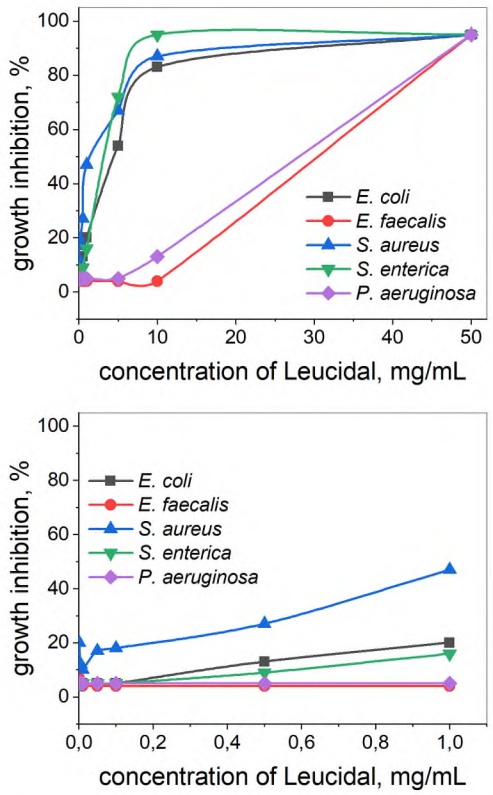

Fig. 1. The inhibition (%) of bacteria growth caused by Leucidal.

the remaining studies (that is *S. aureus* and *E. coli*), it can be concluded that in biological studies the inhibitory effect of Leucidal is stronger on *S. aureus* than on *E. coli*. Additionally, *E. faecalis* and *P. aeruginosa* are the most resistant species (only the highest concentrations of preservative had antibacterial effect).

3.2. The effect of Leucidal on the mixed monolayers imitating bacteria membranes

In Fig. 2 the surface pressure - area (π-A) isotherms for the monolayers mimicking *E. coli* and *S. aureus* membranes spread on a buffer and on Leucidal solutions of different concentrations are shown. In the same figure the compressional modulus (C_S^{-1}) vs the surface pressure (π) plots for the mentioned above systems are presented. The values of the compressional modulus were calculated from the isotherms according to Eq. 1 (Davies and Rideal, 1963)

$$C_S^{-1} = -A\left(\frac{d\pi}{dA}\right)_{p,T} \quad (1)$$

where *A* is the mean area per molecule at the given surface pressure.

In Fig. 3 selected BAM images for these systems are shown.

The isotherms for the monolayer mimicking *E. coli* and *S. aureus* membranes, formed on a buffer, differ from each other in their shape and position. Namely, during compression of the film for *E. coli* model system, the surface pressure starts to increase at ca. 105 Å^2/molecule and it increases monotonically up to ca. 43 mN/m, where a bend in the curve occurs. Then, at ca. 50 mN/m the film collapses. BAM images for this monolayer are uniform in a wide range of the surface pressure, which indicates that the film is homogenous. However, above 35 mN/m the nuclei of the condensed phase are formed and with further compression of the monolayer, the condensed domains become more clearly visible in the images. In agreement with BAM, the maximal value of the compressional modulus at these higher surface pressures is ca. 140 mN/m, which, according to classification provided by Davies and Rideal, indicates a Liquid Condensed (LC) state of the film (Davies and Rideal, 1963).

The morphology of these domains and the surface pressure, at which they are formed correspond well with those observed for one component POPE monolayer (the properties of POPE film will be discussed in the next paragraph).

On the other hand, in the isotherm for *S. aureus* model membrane a plateau region at ca. 12 mN/m appears. Considering the values of the compressional modulus below and above this region (ca. 50 and 250 mN/m, respectively) it can be concluded that the plateau reflects the phase transition between a Liquid Expanded (LE) and Liquid Condensed (LC) state. As it can be seen (Fig. 3) the domains of the condensed phase appear in BAM images even at very low π (at ca. 0.5 mN/m) and they increase in their number with the compression of the film. Above 25 mN/m the images become uniform and the monolayer is in a homogenous condensed phase.

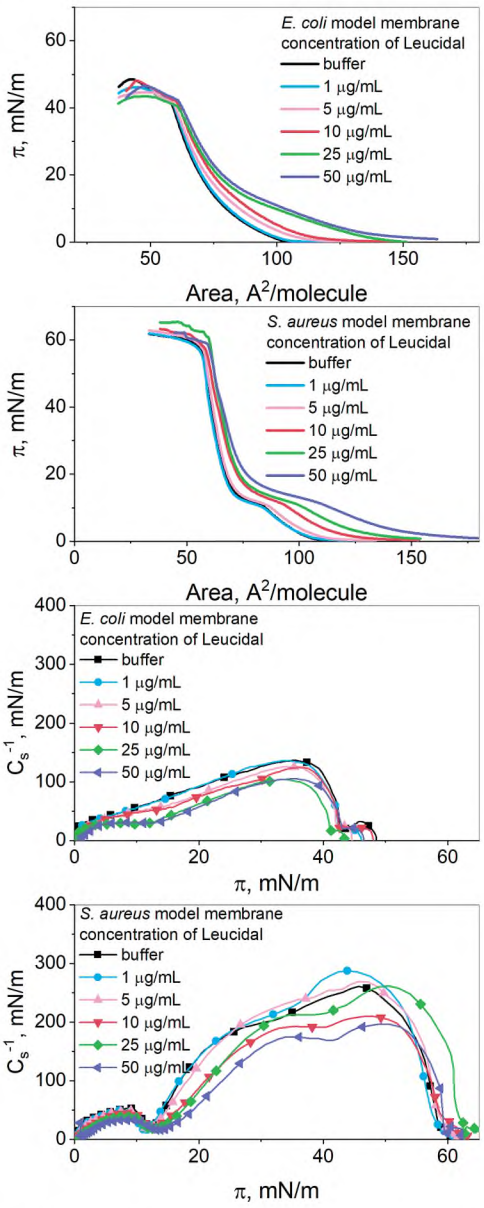

Fig. 2. The isotherms and the compressional modulus vs the surface pressure plots for the mixed monolayers imitating bacteria membranes spread on buffer and on Leucidal solutions.

In general, in the presence of Leucidal, the isotherms for both model systems are noticeably shifted to larger areas, and they become less steep with the increasing concentration of the preservative.

The compressional modulus values in the presence of the preservative are lower as compared to the values obtained for the system on buffer. Only for *S. aureus* membrane at the lowest applied Leucidal concetration some contraction of area is observed (the isotherms are slightly shifted to lower areas). In the case of *S. aureus* membrane also the shift of phase transition to higher surface pressures is observed. It can be summarized that Leucidal causes the model membranes less condensed. The foregoing activity of Leucidal is also observed in BAM images (Fig. 3). In the case of *E. coli* model Leucidal hinders the formation of the condensed domains (namely, on Leucidal solution they appear above 45 mN/m, which is in contrast to ca. 35–40 mN/m for the film on a buffer). Moreover, the domains formed in the presence of Leucidal are visibly smaller than the domains observed for the film on a buffer. In the case of *S. aureus* film, the effect of Leucidal on its morphology is less pronounced however, also for this model the domains

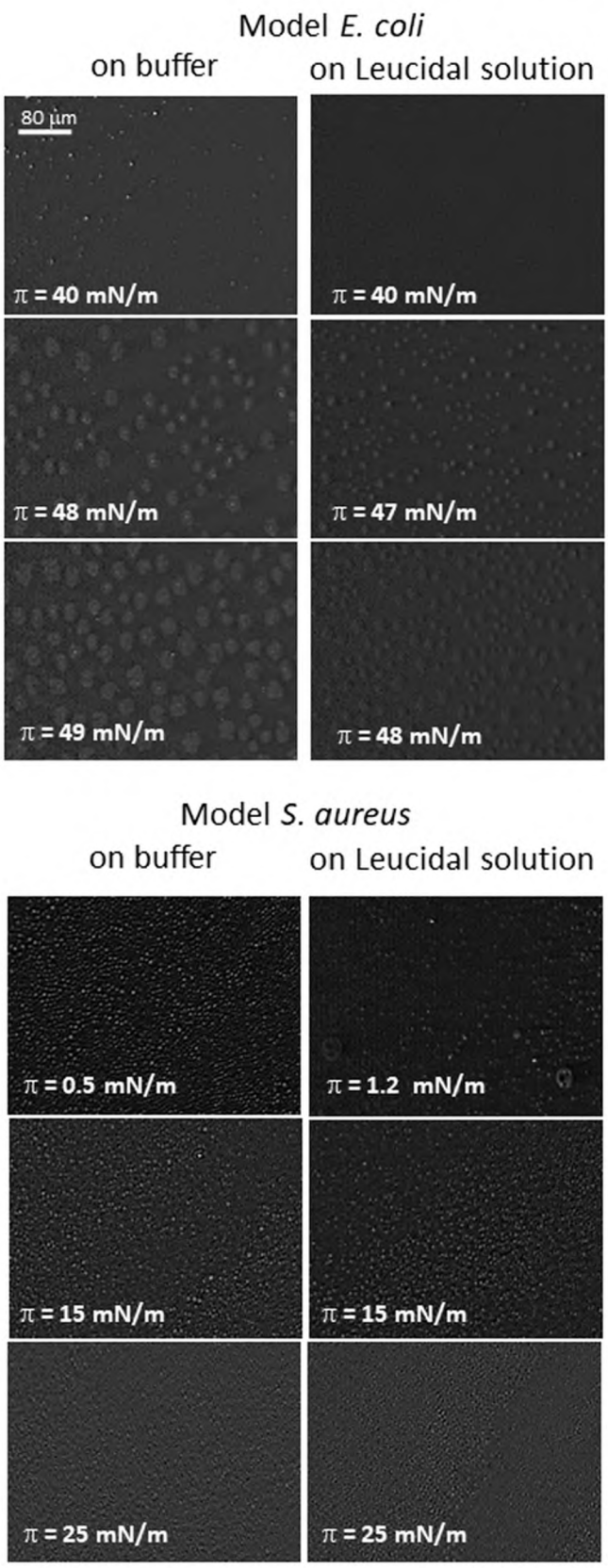

Fig. 3. BAM images for the mixed monolayers imitating bacteria membranes spread on buffer and on Leucidal solution. (The scale bar refers to all images in the Figure).

of the condensed phase are formed at higher surface pressures as compared to the monolayer on a buffer.

Moreover, for both systems, an additional effect of Leucidal is manifested in the isotherms. Namely, at higher preservative concentrations an additional inflection appears (it is well-seen as the minimum in C_S^{-1} vs π plots). For *E. coli* membrane it can be observed at ca. 12 mN/m, while for *S. aureus* film at ca. 40 mN/m. However, it is important to note that at the surface pressures above the inflections both the shift of the curve and the decrease of C_S^{-1} values are still observed. The latter means that the molecules of the studied preservative formulation are still present at the interface and they change monolayer properties. This allows one to conclude that the observed deformation in the isotherm reflects a partial removal of Leucidal from the monolayer rather than phase transition.

To compare deeply the effect of Leucidal on the studied systems, the percentage shift of the curves (in respect to the film on a buffer) caused by the preservative was analyzed (Fig. 4). The calculations were made at 30 mN/m due to a well-known correlations between the properties of the lipids in monolayer and bilayer at these conditions (Marsh, 1996). However, in Supplementary Materials (Fig. S1) the results obtained at $\pi = 10$ mN/m are also presented.

As it can be seen, both at low and high π and in a wide range of concentrations, Leucidal induces very similar effect on the position of the curves for *E. coli* and *S. aureus* model membranes. At 30 mN/m substantial differences (being out of the error range) appear only at two the lowest applied concentrations of the studied formulation. Namely, at these conditions the fluidizing effect of Leucidal is stronger on *E. coli* membrane. This fact may be interesting from the point of view of the anibacterial activity and selectivity of this preservative.

It is also very important that at 30 mN/m the shift of the curves does not change linearly with the concentration of Leucidal (Fig. 4). Namely, initially (at lower concentrations) the lines in Fig. 4 are steep, while at the concentration ≥ 10 μg/mL the lines start to stabilize. This may imply some kind of saturation of the monolayer with Leucidal at these conditions. The latter suggestion seems to be confirmed by the results of calculations at 10 mN/m (Fig. S1). Namely, the differences in the course of these lines at low and higher concentrations of Leucidal are not as evident as they are at higher surface pressure (this is especially pronounced for *S. aureus* membrane). The analysis of the percentage decrease of C_S^{-1} values caused by Leucidal (Fig. 4) evidences that at the lowest applied concentration (1 μg/mL), for both model systems, C_S^{-1} values are, in the error range, comparable with the values on buffer (the decrease of this parameter is ca. 2%). Then, in the range 5 – 25 μg/mL the decrease of C_S^{-1} values for *E. coli* model system is stronger than for *S. aureus* membrane. Finally, at 50 μg/mL, the decrease of this parameter for both model systems is comparable.

In the same figures (Fig. 4 and Fig. S1 Supplementary Materials) the results of penetration experiments are shown. In these experiments different concentrations of Leucidal were used, as indicated in the figures. At 30 mN/m, after injection of Leucidal solution into *E. coli* model system, the surface pressure increases, which means the penetration of the surface active molecules into *E. coli* membrane. The increase of the surface pressure in the studied interval of time does not exceed 2.8 mN/m. Moreover, the results for the concentration 10 μg/mL, that is a slight decrease of the surface pressure in time, evidence that the molecules are removed from the interface in time. For the remaining Leucidal concetrations, in the studied time, the surface pressure stabilizes, which indicates that the surface activive molecules penetrate the lipid environment and stay in the system. The results of penetration experiments correlate well with the finding resulting from the surface pressure-area experiments. Namely, it was found that at higher concentrations and at higher π the preservative is partially removed from the film. In other words, the excess of Leucidal is eliminated from the film, thus the increasing concentration of Leucidal does not lead to its stronger incorporation into the membrane. The results obtained at 10 mN/m confirm the foregoing thesis (Fig. S1). That is, at lower π the

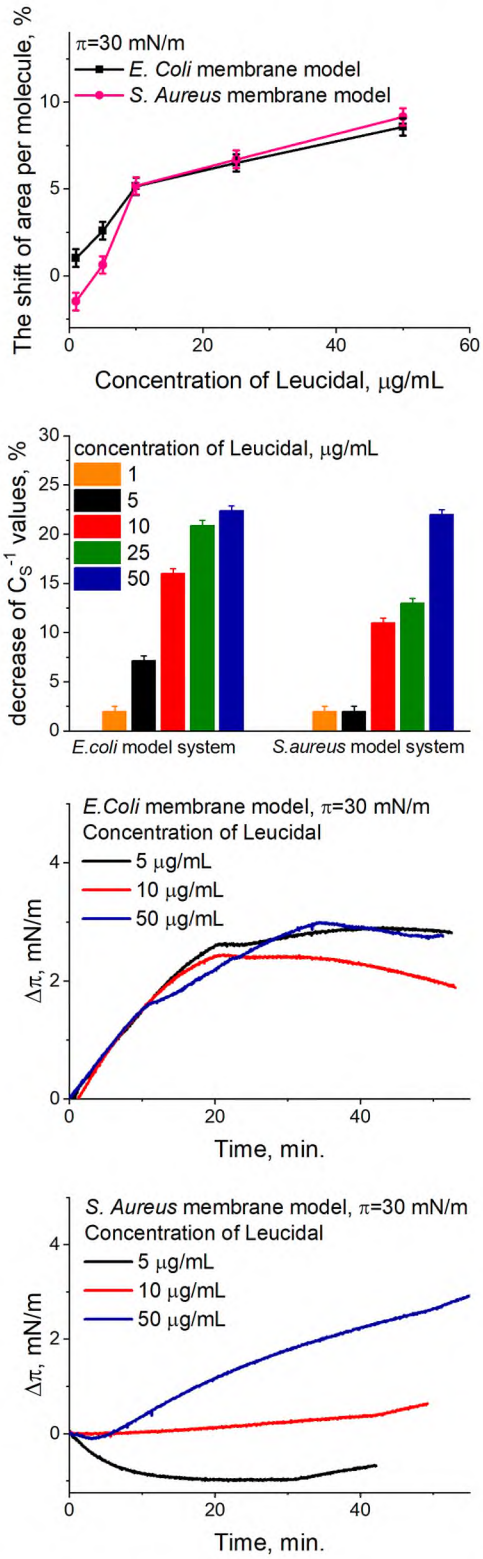

Fig. 4. The shift of the area per molecule values, a decrease of the compressional modulus and the results of penetration experiments at $\pi = 30$ mN/m.

incorporation of Leucidal into *E. coli* membrane increases with the concentration applied ($\Delta\pi$ ca. 7.1 and ca. 8.9 at low and high concentration) and additionally, it is stronger than the penetration at $\pi = 30$ mN/m. This observation can be explained by the fact that at lower surface pressures the monolayers are less condensed, thus the incorporation of membrane-active molecules should be much easier than at

higher pressure region.

In the case of *S. aureus* model membrane, the situation is different. Neither at low nor at high π Leucidal does incorporate into the monolayer at low concentrations. Additionally, at the lowest investigated concentration the surface pressure decreases below π_{in}, which may indicate destabilisation of the film and desorption of molecules from the interface (Preetha et al., 2006). The increase of π resulting from the penetration of the components of the studied formulation into this model was noticed only at the highest applied concentration. Moreover, both at low and high surface pressures the incorporation into *S. aureus* membrane is much weaker as compared to the penetration ability of Leucidal into *E. coli* membrane. To estimate the maximum insertion pressure (MIP) and verify the synergy parameter the additional experiments at 20 mN/m were performed at one Leucidal concetration (50 μg/mL). The Δπ vs π_{in} plots for *E.coli* and *S. aureus* model membranes penetrated by Leucidal in Supplementary Materials (Fig. S2) are shown. The maximum insertion pressure (MIP) for *E. coli* and *S. aureus* were found to be 44.6 and 48.7 mN/m, respectively. This means that the molecules from Leucidal formulation can penetrate the studied model membrane even at very high surface pressures. The synergy is positive both for *E.coli* and *S. aureus* (0.82 and 0.93, respectively), which confirms a favorable binding of the substance to the monolayer (Lhor et al., 2014).

3.3. *The effect of Leucidal on one component lipid monolayers*

In the next step of the investigations, the influence of Leucidal on one-component monolayers formed by the lipids used for the preparation of particular model systems was studied. The isotherms recorded for these films on a buffer and on the subphases containing Leucidal at different concentrations are shown in Fig. S3 and S4 (Supplementary Materials). In the same figures also the C_S^{-1} vs π plots are shown. In Fig. 5 selected BAM images for these monolayers on a buffer and on Leucidal solutions are presented (more images are shown in Supplementary Materials, Fig. S5).

The properties of the monolayers of *E. coli* lipids (POPE, POPG and TOCL) on a buffer were characterized previously in the literature [see e.g (Mach et al., 2021, 2018).]. The isotherms for POPG and TOCL are of a classic liquid expanded character, however, POPG monolayer is more contracted than TOCL film. As it can be found in literature (Pan et al., 2015; Murzyn et al., 2005) the lipid area and the cross-sectional area per oleoyl chain in TOCL bilayers are 129.8 $Å^2$ and 32.5 $Å^2$, respectively while for POPG molecule they are 62.8 $Å^2$ and 31.4 $Å^2$. BAM images for these two films are completely homogenous in a wide range of the surface pressure. However, for POPG film, at very high π values (above 45 mN/m) a phase transition appears and the monolayer becomes condensed (Mach et al., 2018). As far as POPE monolayer is concerned, during compression of this film, above 35 mN/m, a transition to a more condensed state is easily observed. This phase transition manifests itself well as the plateau region in the isotherm, and as the minimum in C_S^{-1} vs π plots as well as in BAM images. In Fig. 5 and S5 (Supplementary Materials) the images for POPE film on buffer are presented. As it can be seen in a wide range of the surface pressure, the images for POPE monolayer reflect a homogenous film. The nuclei of the condensed phase appear above 25 mN/m and during compression their number substantially increases. At ca. 40 mN/m in BAM images the patches of the condensed phase dispersed within liquid matrix are well noticed. With further compression these characteristic domains enlarge and join together and finally at ca. 45 mN/m the monolayer is in a homogenous condensed state.

Cardiolipin used for the preparation of *S. aureus* model system has 4 fully saturated C18:0 chains. Therefore, these molecules are able to form the films of tighter packing as compared to TOCL. This is manifested in lower areas per molecule at a given π and higher C_S^{-1} values for TSCL (C_s^{-1} max ca. 200 mN/m, LC state) films than for TOCL. BAM images taken for TSCL film show that the morphology of the monolayer changes with compression (Fig. 5). Namely, at large molecular areas, the gaseous phase coexists with a more condensed phase. However, with compression, the condensed phase is growing and at 5 mN/m the images are uniform and they reflect the monolayer in a homogenous condensed state.

In the course of the film formed by PG15:0 molecules, a plateau region at ca. 13 mN/m is well noticed. The compressional modulus values below and above the plateau (37 and 230 mN/m, respectively) evidence LE – LC phase transition. This transition reflects also in BAM images for this film (Fig. 5). Namely, up to ca. 12 mN/m the images are dark and homogenous, while at 13 mN/m the domains of the condensed phase appear. With further compression, these domains enlarge in the number and size and finally at $\pi > 40$ mN/m they cover the interface and the film is homogenous and in LC state.

In the presence of Leucidal the general trend is that the isotherms for one-component lipid films are shifted to larger areas as compared to the monolayers on a buffer and additionally, the compressional modulus values drop. However, for TSCL film compressed in the presence of Leucidal at the lowest applied concentration (1 μg/mL) the curve is slightly shifted to smaller molecular areas and the C_S^{-1} values are slightly higher as compared to the monolayer on buffer. Additionally, for PG15:0 film the isotherm recorded on the lowest applied concentration of Leucidal (1 μg/mL) and the isotherm for the film on buffer are nearly identical. Moreover, for this films (PG15:0) at high surface pressures (above 40 mN/m) and for all preservative concentrations used in experiments, C_S^{-1} values increase significantly above the values for the monolayer on buffer.

Moreover, at higher concentrations of Leucidal in the subphase, in the isotherms for one component lipid films, the characteristic deformation can be noticed. Similar effect was observed for the mixed systems imitating *E. coli* and *S. aureus* membranes and it can be attributed to the exclusion of the molecules of the preservative formulation from the film. As it can be observed in Fig. 5 Leucidal modifies also the morphology of the films making them visibly less condensed as compared to the monolayers on a buffer.

The comparative analysis of the effect of Leucidal on one component monolayers of the lipids forming model membranes was made based on the calculated percentage shifts of the curves and the percentage drop of C_S^{-1} values, (at $\pi = 30$ mN/m). The results are shown in Fig. 6 together with the data for the mixed systems presented previously.

Considering the results for *E. coli* lipids, practically in the whole range of the concentrations applied, Leucidal exerts the strongest effect on the position of the curve for POPG monolayers. The effect on POPE and TOCL film can be described as comparable. Summarizing, Leucidal makes one component lipid monolayer less condensed in the following way: POPG>POPE≈TOCL. Considering the effect of the preservative on C_S^{-1} values it can be concluded that Leucidal decreases the values of this parameter in the following way: POPG≈TOCL>mixture (*E. coli* model system)>POPE.

For *S. aureus* lipid films the differences in the effect of Leucidal applied in higher concentrations on the model system and on one component lipid films are less pronounced and it can be stated that the effect of this substance on the position of all the curves, is very comparable. At lower Leucidal concentrations the differences are more pronounced and the strongest shift to larger areas appears for PG15:0 monolayer, while the influence on TSCL and model system are very similar. However, more pronounced differences appear when the C_S^{-1} drop is analyzed. Namely, Leucidal exerts definitely stronger effect on the values of this parameter for PG15:0 monolayers in comparison with TSCL film. Its influence on the mixed film (that is *S. aureus* model membrane) is intermediate between the effect on both components of the mixture.

Interestingly, the strongest drop in C_S^{-1} values is observed for both studied herein PGs monolayers. This may indicate that the structure of PG polar head and the negative charge may be of importance as regards Leucidal affinity. On the other hand, also cardiolipins carry the negative

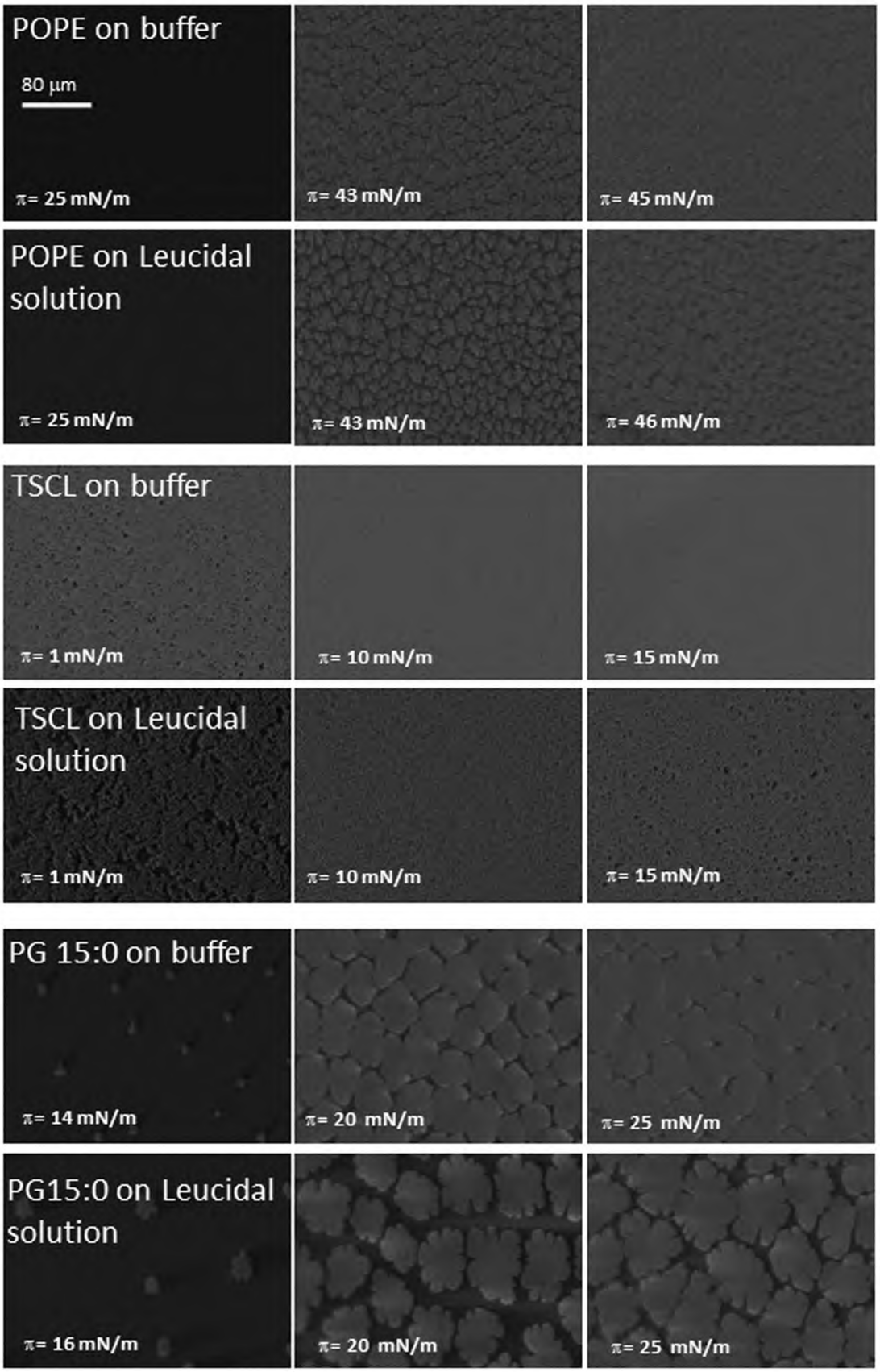

Fig. 5. BAM images for one component lipid monolayers on buffer and on Leucidal solution. (The scale bar refers to all images in the Figure).

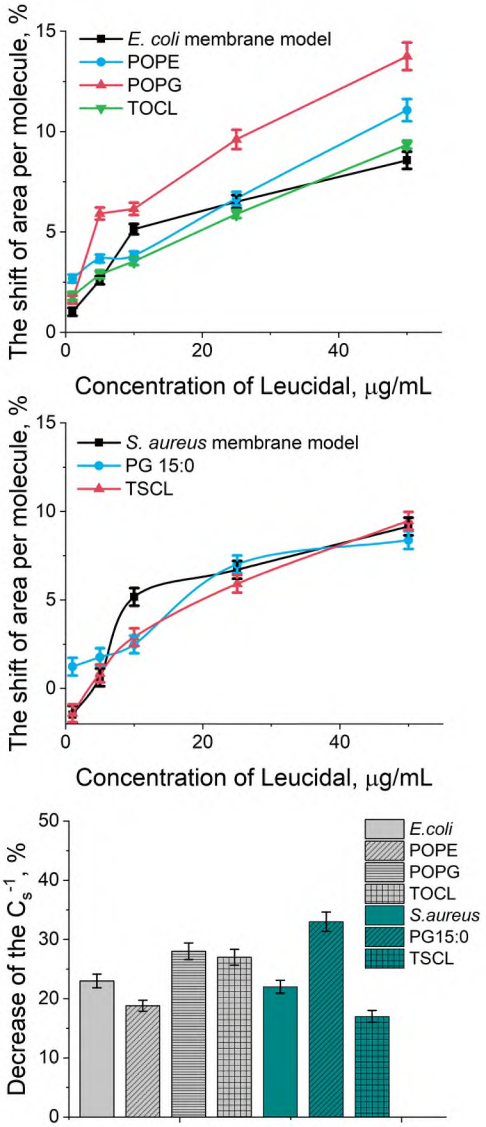

Fig. 6. The shift of the area per molecule values (at $\pi = 30$ mN/m) and a decrease of the compressional modulus values (at $\pi = 30$ mN/m and Leucidal concentration 50 μg/mL) for one component lipid monolayers spread in the presence of Leucidal.

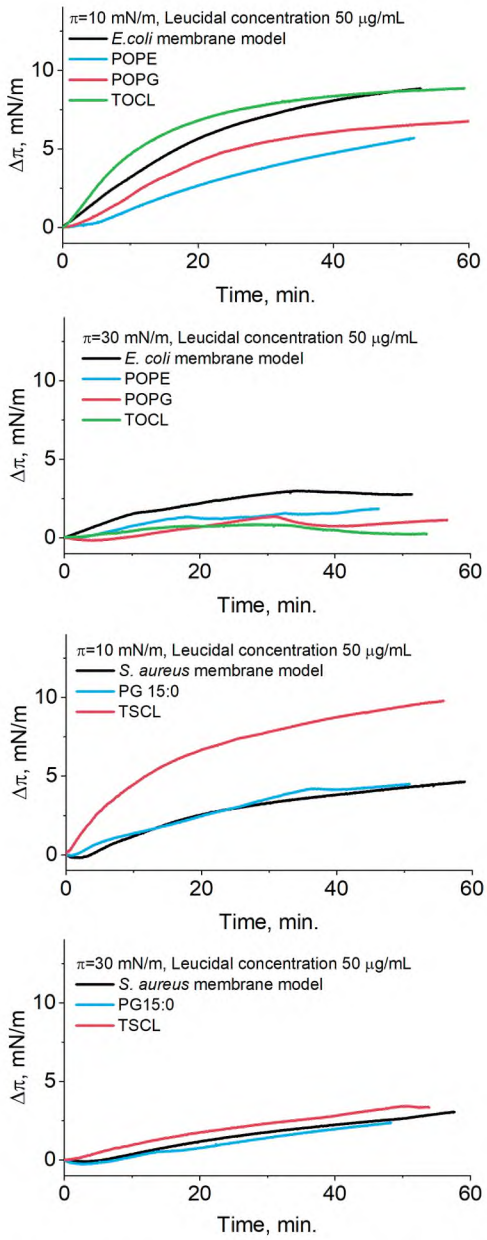

Fig. 7. The results of penetration studies of Leucidal into lipid monolayers.

charge however, only for TOCL the decrease in C_S^{-1} value is comparable to the drop observed for POPG. These findings may suggest that the effect of Leucidal components on lipid films is not determined solely by electrostatics, but that the structure of the monolayer (that is, its molecular organization) is also important.

The results of penetration experiments performed at 10 and 30 mN/m are shown in Fig. 7. First of all, the penetration of Leucidal is stronger at a lower surface pressure. Moreover, at a lower surface pressure, the penetration is the strongest for both CLs, then for both PGs and finally for POPE monolayer. At 30 mN/m the differences in the increase of π are not as pronounced and in fact it is impossible to indicate the one – component lipid film, which is preferentially penetrated by the studied preservative fomulation.

Considering the results for the lipids of *E. coli* membrane the penetration ability of Leucidal at 10 mN/m decreases in the order: TOCL>POPG>POPE. The incorporation of Leucidal into mixed system and TOCL film is very comparable, although immediately after injection stronger incorporation into TOCL than into the mixture can be found. This is very interesting because it seems that TOCL, which is definitely a minor component of the mixed model system, strongly determines the affinity of Leucidal to this film. At $\pi = 30$ mN/m the penetration of Leucidal into *E. coli* model membrane is stronger than into the respective one component lipid films. Thus, it can be concluded that at higher π, when the film is more densely packed than at 10 mN/m, the organisation of lipids in a mixture ensures more favourable conditions for penetration than individual lipids do.

For the lipids of *S. aureus* model membrane, the collected results evidenced that at low surface pressure, the incorporation is the strongest for cardiolipin monolayer, and it is very comparable for PG and the mixed film. At high surface pressure the observed differences between the curves are minimal.

4. Discussion

The in vitro experiments confirmed bacteriostatic activity of Leucidal formulation against the tested bacteria. Gram-positive *S. aureus* bacteria and Gram-negative *E. coli* bacteria were those, against which the inhibitory effect of Leucidal was the strongest (*S. aureus*>*E. coli*) over

almost the entire range of the concentrations tested. To compare the effect of Leucidal on bacteria membranes two mixed lipid monolayers imitating *E. coli* and *S. aureus* membranes were investigated. It was found that the components of the preservative formulation incorporate into the monolayers and they change their morphology and organisation. Interestingly, at higher concentrations, the molecules were partially removed from the films. The latter manifests itself well at 30 mN/m, but not at 10 mN/m. Therefore, it can be proposed that the packing of the lipids increasing with the compression of the film makes the incorporation of the molecules from the subphase more difficult. These results suggest additionally that the use of Leucidal at higher doses does not provoke its stronger effect on the membrane. It was also evidenced that the differences in the effect of Leucidal on both model systems appear only at low concentrations of the preservative. Namely, up to the concentration 5 μg/mL, the influence of Leucidal is stronger on *E. coli* membrane. A stronger affinity of Leucidal to *E. coli* membrane was also found in the penetration experiments.

Since the studied model membrane systems differed in their composition, the effect of Leucidal on the films formed by the individual lipids was also investigated. Firstly, it was found that qualitatively the influence of Leucidal on one component lipid films is similar to its effect on the mixed systems. That is, the preservative decreases the condensation of the monolayers and modifies their morphology. Moreover, for all these films also the partial exclusion of the Leucidal molecules from the monolayers can be postulated. Secondly, it was found that, generally, Leucidal exerts a stronger effect and has a stronger affinity to PGs and cardiolipins and among them to the lipids used for the preparation of *E. coli* model system. However, the latter does not correlate with the finding on rather similar effect (in a wide range of the concentrations applied) of Leucidal on *E. coli* and *S. aureus* membranes. On the other hand, these results seem to be reasonable taking into account the composition of the mixed systems. Namely, in *E. coli* membrane POPG and TOCL are in significantly lower level (25%) than POPE (75%). Thus, the lipids the most strongly responsive to Leucidal are in definitely lower content than POPE, which forms monolayers the least sensitive to the preservative.

To analyze the effects, which may govern the influence of Leucidal on the lipid membranes more deeply it is necessary to refer to the composition of the studied formulation. As it was mentioned in the Introduction the producer of Leucidal® Liquid informs that this formulation contains a novel antimicrobial peptide as well as salicylic acid, while the other analysis of commercial Leuconostoc/radish root filtrates confirmed the presence of didecyldimethylammonium salt (chloride) as well, however, no peptide was detected (Technical Dossier Leucidal® Liquid, 2022; Li et al., 2015). All these substances are known from their antimicrobial properties. As regards AMPs, they are usually amphipathic positively charged molecules active against bacteria, fungi and even viruses. They differ each other as regards the surface activity, which depends on the structural factors (e.g. molecular size, conformation, flexibility, net charge of peptide) and on the experimental conditions (ionic strength, pH, temperature of the bulk phase as well as the peptide concentration) (Barzyk et al., 2013; Maget-Dana, 1999). It is also very important that the affinity of a peptide to the monolayer covering the surface does not necessarily correlate with the surface activity of the peptide molecule. As it was demonstrated in the penetration studies (Barzyk et al., 2013) two peptide molecules can exhibit strong and comparable affinity for the tested monolayer despite the fact that one of them is surface active at the air/water interface, while the second does not display the surface activity. This is due to the differences in the conformation of the peptide molecules in the solution bulk and in the lipidic environment (Barzyk et al., 2013). The mechanism of antimicrobial action of peptides is very complex. Some of them act by a direct interactions with membrane components, the other mainly by electrostatic forces with the negatively charged parts of membrane; they can be also able to induce membrane pore formation or to extract the membrane lipids out. Summarizing, their activity is membrane-related that is they may cause changes in membrane structure and function and causing even the cell membrane damage or they may enter the cell interior via membrane and act on intracellular components (Herman, 2019; Ageitos et al., 2017; Seyfi et al., 2020; Travkova et al., 2017; Zhang et al., 2021; Agadi et al., 2022).

Didecyldimethylammonium chloride (DDAC), similarly to antimicrobial peptide, is a positively charged molecule. This compound is also surface active and displays Critical Micelle Concentration (CMC) value: 1.2 mM (0.434 mg/mL) at 25 °C (the adsorption curve can be found in literature e.g (Leclercq and Nardello-Rataj, 2022).). However, the surface activity of this compound may change in the presence of other substances in the system [see e.g. 37]. DDAC is a well-known disinfectant having antibacterial and antifungal properties (Jansen et al., 2013). It is also membrane-active and causes the leakage of important intracellular material and membrane disruption (Jansen et al., 2013; Yoshimatsu and Hiyama, 2007). This compound has also potential for development of skin irritation and hypersensitivity after dermal exposure (Anderson et al., 2016).

And finally salicylic acid is a preservative used in food industry, which is active against bacteria (including *E. coli* and *S. aureus*) and fungi. In a wide range of pH, it carries a negative charge ($pK_{a1} = 3.0$, $pK_{a2} = 13.4$) (Minczewski and Marczenko, 2001; Bernal et al., 2018). Although salicylic acid molecules are rather small and lack the characteristic amphiphatic structure, as results from literature (Dynarowicz et al., 1988), they are able to adsorb from solutions at the free surface of water. It was also evidenced that salicylic acid in mixture(s) with other compounds influences their surface activity (Cid et al., 2014). Various mechanisms of antimicrobial action of salicylic acid were proposed, including: the disruption of bacteria cell wall and membrane leading to the leakage of intracellular alkaline phosphatases, nucleic acids and proteins; downregulation of some of the virulence factors in bacteria and reduction of the production of extracellular polysaccharides by bacteria (Bandara et al., 2006; Pereira et al., 2018; Song et al., 2022).

Since Leucidal is a mixture of the compounds, the properties of this formulation, that is the surface and membrane activity as well as antimicrobial effect, are determined by the properties of their components and their mutual interactions.

In addition to the properties of Leucidal mixture, the effect of this preservative on membranes is also influenced by the properties of the lipids forming monolayers. Namely, the positively charged components of the preservative formulation have affinity to the negatively charged lipids. However, it is not only electrostatic interactions that should be considered here. The organization of the monolayer is also the factor determining the incorporation of Leucidal components into model systems. In this context also the packing defects should be mentioned. Packing defects, defined as the regions of a decreased density of lipid molecules, are formed in the consequence of membrane bending or, in a flat membranes, as the results of the presence of cone-shaped lipids with a small headgroup (for example PE, cardiolipin). The presence of unsaturated chain(s) in the lipid molecules also promotes the defects formation. Packing defects were indicated as the factors promoting the binding of proteins to membranes by forming spaces between lipid head groups (Sugiura et al., 2021; Pinot et al., 2021; Sikdar et al., 2022; Bigay and Antonny, 2012). In a simple words the packing defects facilitate the incorporation of molecules into the membrane.

The significance of the summarized above factors regulating the membrane–bioactive molecules interactions are confirmed by the results of the experiments performed by using the lipid Langmuir films as model of bacteria membranes. For example in the studies on the effect of antimicrobial peptide on DPPG/TMCL and DPPG/DPPE monolayer it was found that the investigated AMP molecule binds more strongly and penetrates more effectively the system composed of the negatively charged lipids and being more loosely packed (DPPG/TMCL monolayer). The latter indicates on the significance of peptide/lipids electrostatic interactions as well as on membrane condensation (Ciumac et al., 2021). However, the same investigations (Ciumac et al., 2021)

evidenced that the penetration of peptide was very comparable for TMCL and DPPG, which are structurally different molecules, and it was noticeably weaker into DPPE. This confirms that for one components lipid system the main factor driving AMP – lipid binding is electrostatics. Moreover, similarly to our results also in these studies the collected MIP values were very high, however, the penetration of peptide was stronger as compared to the penetration detected for the formulation studied by us (higher $\Delta\pi$ values at a given initial surface pressure). On the other hand, the ability of selected AMPs derived from bovine milk to penetrate DPPG monolayer was more comparable to the results obtained for Leucidal and moreover, for these peptides their removal from the monolayer was evidenced, similarly to our findings (Barzyk et al., 2013). In another experiments (Clausell et al., 2007) the Langmuir films composed of lipopolysaccharide (LPS) or POPG imitating outer and inner membrane of bacteria cells were used to study the mechanism of action of cationic amphiphatic AMPs (polymyxin B and its derivatives). It was found for example that one of derivatives of the native compound inserts easily into LPS film but does not penetrates into POPG monolayer at the same surface pressure and concentration, although both these molecules are negatively charged. It was confirmed that this derivative binds interfacially to the polar heads of PG molecules, without insertion into the monolayer. The results indicated that in this peptide – lipid interactions both electrostatic forces and hydrophobic domains of peptide molecules are involved as well as that the peptide activity is determined by the cationic amphipathicity (Clausell et al., 2007). The Langmuir monolayer experiments were also applied to discuss the relationship between the structure of polar head and hydrophobic tails of phospholipids and the effect of antimicrobial peptide [see e.g. 54]. It was shown that AMP incorporates more readily into anionic monolayers (PG and lipid A layers); that among zwitterionic layers it prefers phosphatidylcholines over PEs, and that the increased packing density of the monolayer (due to increased pi or saturation of the tails) impairs the insertion of peptide. Most importantly, the results presented confirmed the significant role of the lipid composition of membrane in AMP activity (Ishitsuka et al., 2006).

By comparing the collected herein results of the studies in model systems with the results of biological tests even more valuable information about Leucidal's activity were obtained. *In vitro* tests evidenced that Leucidal exerts stronger inhibitory effect against *S. aureus* bacteria as compared to *E. coli* strain. This is in contrast to the results of the studies on model membranes. Therefore, it can be suggested that the cell membrane is probably not the main site of action of the active substances contained in the tested product. At this point, it should be noted that the outermost part of the cell is the cell wall, which is characterized by a large diversity among bacteria and significantly affects the resistance of bacteria to external physical and chemical factors.

Moreover, high concentration of the tested preparation shows antibacterial activity in relation to all tested bacteria. This may suggest a lack of selective action, which is a desirable feature in the case of an additive designed to protect the product against microbiological contamination. The selectivity of the components of cosmetics is also important from the point of view of a proper functioning of the skin microbiome. It is well-known that human skin is covered by various bacteria and fungi forming ecosystem, which protects from harmful effects of UV rays, pollution, pathogenic microorganisms and ensures health of skin. The components of cosmetics may change the balance of the skin microbiota and the experimental data indicate that preservatives are of special role from the point of view of the skin microbiota dynamics (Pinto et al., 2021).

To compare the antimicrobial potency of the studied herein formulation with the properties of other preservatives in Table S2 (Supplementary Materials) the values of MIC and/or MBC presented in literature for selected cosmetic preservatives were compiled (Kamysz and Turecka, 2005; Adamczak et al., 2019; Mirsonbol et al., 2014). It is clear that the experimental procedure strongly determines the values of MIC/MBC. However, analyzing the information presented in Table S2 it can be concluded that antibacterial potency of presented antimicrobial peptide preservatives (Citropin 1.1 and Protegrin 1) as well as the remaining widely used cosmetic preservatives (including parabene and acids) is stronger as compared to the effect found for Leucidal. This fact does not exclude the possibility of the use of Leucidal as preservative, however, to achieve the desired protective effect, this substance should be applied in higher concentrations than the remaining preservatives. Moreover, more experiments (including also toxicity of this product) should be performed to explore the effect of this formulation on cells.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Data Availability

Data will be made available on request.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.chemphyslip.2023.105338.

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