Surfactants – the application in pharmaceutical biocatalysis

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Surfactants – the application in pharmaceutical biocatalysis

Biocatalysis is one of the most commonly applied processes using enzymes. The modulation of biocatalytic reactions often requires the addition of molecules with various tendencies to behave in aqueous and/or non-aqueous media. Surfactants, as compounds containing both hydrophilic and hydrophobic groups in their structure, seem to be extremely useful in biotechnological, chemical, and pharmaceutical industries. Surfactants can be divided based on their e.g. electrical charge (cationic, anionic, amphoteric, non-ionic) and/or chemical structure (cyclic, acyclic). These compounds were applied especially in reactions catalyzed by lipases, the enzymes characterized by high activity and stability. Thus, lipases, due to their unique properties (interfacial activation), are widely used in reactions with pharmaceutical significance.

This review aimed to show the effect of surfactants on lipase activity, especially in various pharmaceutical reactions such as obtaining drugs or their building blocks. The participation of surfactants in the reactions catalyzed by 12 various lipases – lipase from *Candida rugosa* (CRL), lipase B from *Candida antarctica* (CALB), lipase from *Burkholderia cepacia* (BCL), *Thermomyces lanuginosus* (TLL), *Pseudomonas fluorescens* (PFL), *Pseudomonas aeruginosa* (PAL), *Pseudomonas stutzeri* (PSL), *Aspergillus oryzae* (AOL), *Aspergillus niger* (ANL), *Yarrowia lipolytica* (YLL), *Rhizomucor miehei* (RML) and *Fusarium oxysporum* (FOL), has been described.

The literature data showed the effect of applied surfactants on lipase activity. The positive effect of non-ionic Tween (20, 80) on the activity of most used lipases (CRL, CALB, BCL, PAL, PSL, AOL, ANL) has been presented. The application of non-ionic Triton X-100 in the reaction mixture has also beneficial impact on lipase activity (CALB, BCL, TLL, PFL, PSL, AOL, RML, FOL). On the other hand, ionic surfactants such as sodium dodecyl sulfate (anionic, SDS) showed various effects – the increase of RML activity, and decrease in CALB, TLL, PFL, and PSL activity have been observed. In turn, the cetyltrimethylammonium bromide (cationic, CTAB/CTABr) positively influenced BCL, AOL, and ANL and negatively on CRL, TLL, PFL, PSL,

and RML lipase activity. The effect of surfactant on lipase activity was dependent on the detergent structure and concentration and reaction conditions e.g. pH, and temperature. Therefore, surfactants are considered important components in developing catalytic systems.

Keywords: biocatalysis, surfactants, lipase, lipase activity.

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List of abbreviations

3H3P	–	3-hydroxy-3-phenylpropanonitrile
(*S*)-3H3P	–	(*S*)-3-hydroxy-3-phenylpropanonitrile
ANL	–	lipase from *Aspergillus niger*
AOL	–	lipase from *Aspergillus oryzae*
AOTNa	–	Bis(2-ethylhexyl)sodium sulfosuccinate
BCL	–	lipase from *Burkholderia cepacia*
BNO	–	Brazil nut oil
C	–	conversion
CALB	–	lipase B from *Candida antarctica*
CB1-12	–	dodecyldimethylammonium methanecarboxylate
mCLEAs	–	magnetic cross-linked enzyme aggregates
CMC	–	critical micellar concentration
CRL	–	lipase from *Candida rugosa*
CRL-OF	–	lipase OF from *Candida rugosa*
CTAB/CTABr	–	cetyltrimethylammonium bromide
*p*DoAO	–	*p*-dodecyloxybenzyldimethylamine N-oxide
EPROS	–	enzyme precipitated and rinsed with organic solvents
FAA	–	fatty acid amides
FOL	–	lipase from *Fusarium oxysporum*
HAP	–	hydroxyapatite
IPC-SDS	–	sodium lauryl sulphate
MFs	–	microflowers
MNPs	–	magnetic nanoparticles
MOPE	–	(*R*)-1-(4-methoxyphenyl)-ethanol
NaTDC	–	sodium taurodeoxycholate
pNPA	–	*p*-nitrophenyl acetate
pNPB	–	*p*-nitrophenyl butyrate
pNPP	–	*p*-nitrophenyl palmitate
OG	–	*n*-octyl-β-D-glucopyranoside
PAL	–	lipase from *Pseudomonas aeruginosa*
PCL	–	lipase from *Pseudomonas cepacia*
PEG	–	polyoxyethylene glycol
PFL	–	lipase from *Pseudomonas fluorescens*
PSL	–	lipase from *Pseudomonas stutzeri*
RML	–	lipase from *Rhizomucor miehei*
SB3-12	–	dodecyldimethylammonium propanesulfonate
SDS	–	sodium dodecyl sulfate
SL	–	structural lipid
TLL	–	lipase from *Thermomyces lanuginosus*
TTAB	–	tetradecyl trimethylammonium bromide
YLL	–	lipase from *Yarrowia lipolytica*

Introduction

Hydrophilicity and hydrophobicity are some of the most important features of chemical compounds. Due to these properties, hydrophilic substances show activity in an aqueous medium, while hydrophobic substances do so in a non-aqueous one. When designing a reaction system, it is often required to use a two- or multi-phase system comprising both an aqueous and a non-aqueous phase. Surfactants, due to their unique structure (amphiphilic character) consisting of a hydrophilic head and a hydrophobic "tail", show surface activity in polar and nonpolar media. In a hydrophilic medium, these compounds are adsorbed on the phase border, decreasing its tension. When the surfactant concentration exceeds the critical value - critical micellar concentration (CMC), the aggregates, or micelles, are formed. In water, this structure is oriented with the hydrophilic part toward the reaction medium. However, the structure of micelles is addicted to molecule geometry. Additionally, external factors such as temperature and the presence of electrolytes can also modify the type of formed structures. Surfactants can be divided into 4 main groups:

a) cationic–surface activity determined by cationic group (e.g., sulfates, sulphonates, carboxylates);
b) anionic–surface activity determined by anionic group (e.g., amines and their salts, acylated diamines, and polyamines);
c) amphoteric (zwitterions) – surface activity determined by the cationic and anionic group, according to the pH of the medium (e.g., aminoamides, sulfobetaine);
d) non-ionic – without electronic dissociation (e.g., etoxylates, polymers, glycerol esters).

The application of surfactants, due to their amphiphilic character, in the pharmaceutical, medical, and biotechnological industries is commonly known. An interesting example of reactions using surfactants is biocatalysis, i.e., chemical reactions catalyzed by enzymes. Biocatalytic processes, due to their environmental friendliness, high efficiency, and low cost, are gaining more and more interest among researchers. Particularly involved in biocatalysis reactions are lipases (EC 3.1.1.3), i.e., enzymes from the group of hydrolases. Thanks to the presence of a lid in most lipases, the activation of the water-oil interface is possible.

According to Verma *et al.* [1], the catalytic activity of lipase is interfacial activated due to the reaction enzyme existing on the substrate at the micellar interface, increasing the turnover number concerning an enzyme concentration scale. These enzymes are either applied in free form or undergo

modification of their structure due to interactions with physicochemical materials. The main example of this process is immobilization. Therefore, lipases, due to their properties, are commonly used in many reactions with pharmaceutical and biotechnological significance, such as obtaining optically pure enantiomers of racemic mixtures of drugs or their building blocks and achieving ecologically friendly biodiesel. The practical application of lipase often occurs with the addition of surfactants. Both lipases and surfactants act at the interface of the reaction medium. The concentration of surfactant (especially the ionic type) influences lipase hydrophobicity and stability.

The aim of this work is to present a review of the effect of surfactants on the activity of various lipases and their application in pharmaceutical biocatalysis.

Methodology

In the preparation of this publication, the Clarivate search engine was used, using the Web of Science database. The literature review included publications from the last 10 years (2014–2023). Older articles were cited only when there were no newer reports covering the subject of the review and when the authors considered them to be an extremely valuable source of information, necessary for the substantive value of the work. The following keywords were used to search for publications: *lipase*, *surfactants*, *lipases application* and *surfactants application*. Among the found articles, those that concerned the use of lipases in the pharmaceutical industry and the influence of surfactants on the activity of lipases were selected. The number of publications searched by keywords: *lipase and surfactants* found between 2014–2023 is presented in Figure 1. The division of references, according to the release year is presented in Figure 2.

Lipase from *Candida rugosa* (CRL)

Lipase from *Candida rugosa* (CRL) belongs to the most commonly applied enzymes in reactions with the presence of surfactants. CRL is characterized by high enzymatic activity and wide substrate specificity. The application of CRL in pharmacy includes obtaining chirally pure active compounds

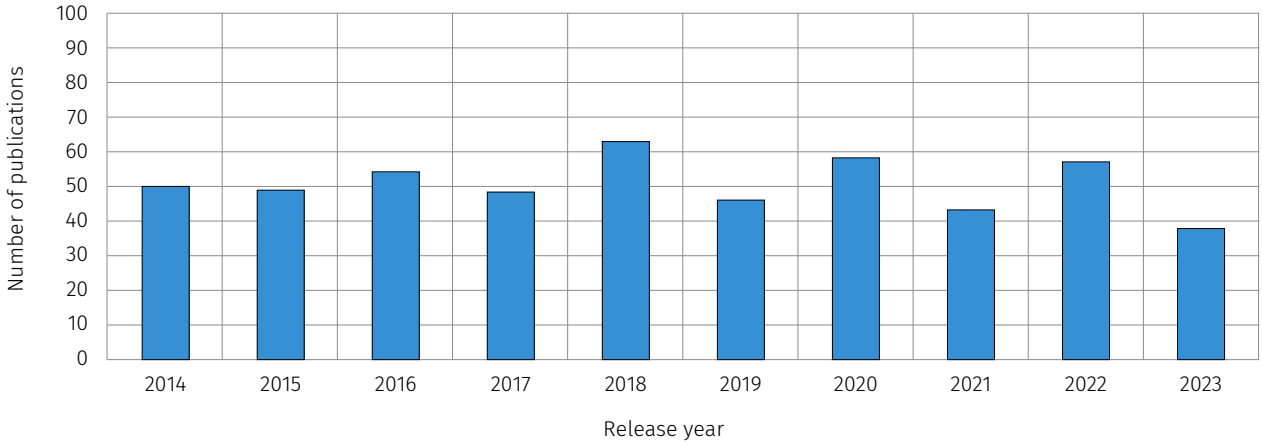

Figure 1. The number of available publications searched by keywords: *lipase and surfactants* between 2014–2023 (n = 506).

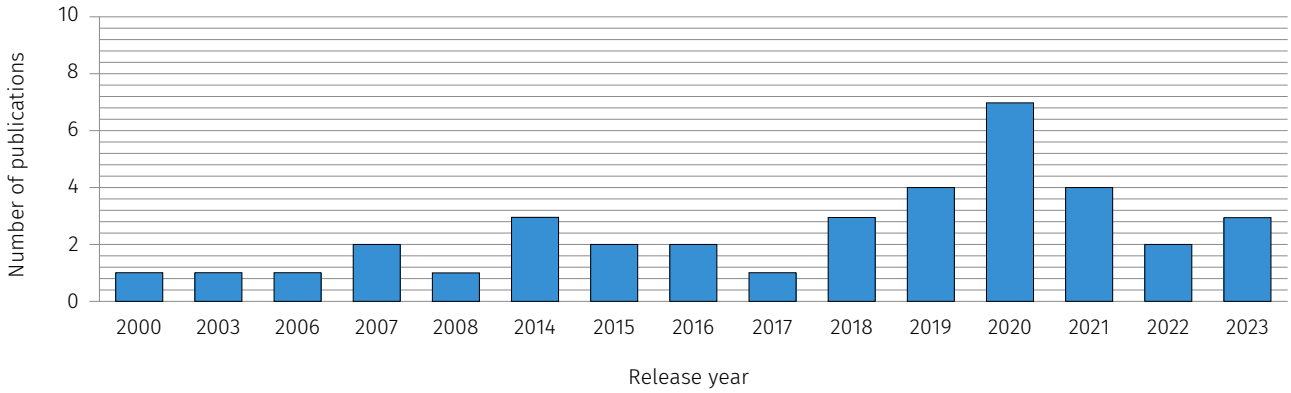

Figure 2. The division of references, according to the release year (n = 37).

being enantiomers of a racemic mixture of drugs e.g., ibuprofen, ketoprofen, or atenolol. Therefore, the reaction catalyzed by CRL with the addition of surfactants is developed. Gabriele *et al.* [2] studied the effect of cationic, anionic, and zwitterionic surfactant structures on CRL activity. The sodium dodecyl sulfate (SDS) as anionic and cetyltrimethylammonium bromide (CTABr/CTAB) as cationic surfactants were tested to evaluate the effect of head group of surfactant charge on CRL enzymatic activity. In turn, the dodecyldimethylammonium methanecarboxylate (CB1-12), dodecyldimethylammonium propanesulfonate (SB3-12) (concentration of, and *p*-dodecyloxybenzyldimethylamine N-oxide (*p*DoAO) in the concentration of were investigated as the zwitterionic compounds. The structures of used surfactants are presented in Figure 3 and the concentration of used detergents were shown in Table 1. The assessment of CRL lipase was performed due to the hydrolysis of *p*-nitrophenyl acetate (*p*NPA). Based on the obtained results, the activity of CRL using the SDS increased to the concentration of 0.002 M, after which there was a decline, but activity remained at a high level (> 1.0). In the case of CTABr, the lipase activity significantly decreased. However, further addition of surfactant did not affect enzyme activity. The authors concluded that the lengthening of the alkyl chain of surfactant improved the catalytic activity of CRL. On the other hand, with the growth of surfactant head size, the CRL activity decreased. In a different study [3], the SDS and L-proline belonging to the acyclic surfactant, and the *p*-sulfonatocalix[4]arene, *p*-sulfonatocalix[8]arene, and calix[4]arene L-proline derivatives (Figure 4) as cyclic compounds were used in various concentrations (Table 1) to study the effect on CRL activity. The evaluation of lipase activity was measured as a hydrolyze of *p*-nitrophenyl palmitate (*p*-NPP). In the case of anionic surfactants, the obtained parameters (relative activity) showed hyperactivation of CRL in a reaction with the use of 1.25-5 mM *p*-sulfonatocalix[8]arene or *p*-sulfonatocalix[4]arene. On the other hand, the high activity of CRL was observed in the presence of calix[4]-L-proline in the concentration of 0.625-5 mM. The obtained results showed a positive effect of cyclic surfactants on CRL activity. The authors stated that the cyclic structure of anionic surfactant could create micelles and complexes with cationic structures and amino acid residues in the enzyme. According to the zwitterions properties, the protection of the enzyme's

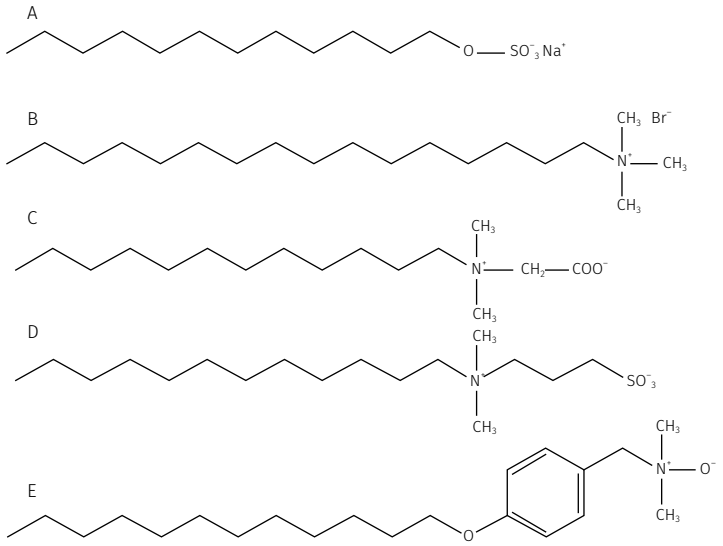

Figure 3. The structures of surfactants affecting CRL activity [2]: SDS (A), CTABr/CTAB (B), CB1-12 (C), SB3-12 (D), *p*DoAO (E).

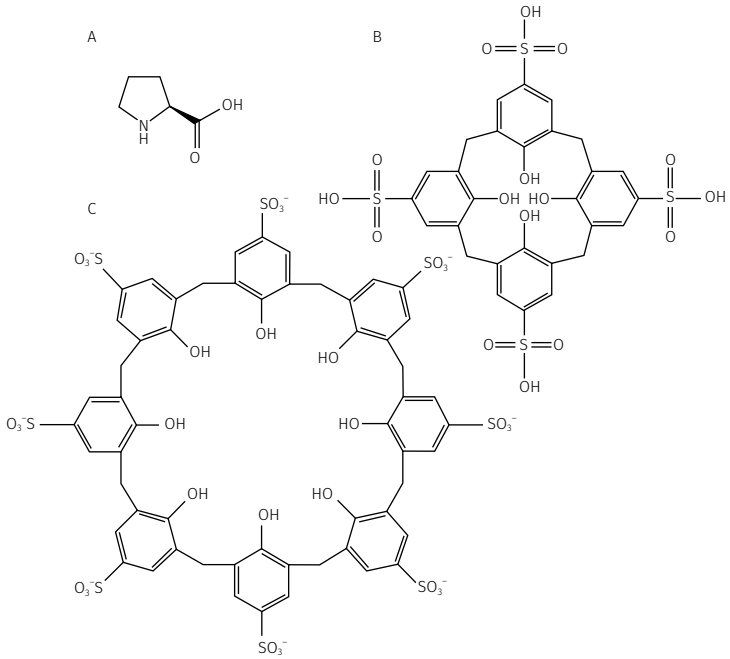

Figure 4. The structures of L-proline (A) and the cyclic surfactants [3]: the *p*-sulfonatocalix[4]arene (B), *p*-sulfonatocalix[8]arene (C).

Table 1. The concentrations of surfactants tested in the reactions catalyzed by CRL.

Surfactant	SDS	CTABr	CB1-12	SB3-12	*p*DoAO	Reference
Concentration range [M]	≤ 0.01		≤ 0.01		≤ 0.008	[2]
Surfactant	***p*-sulfonatocalix[4]arene**	***p*-sulfonatocalix[8]arene**	**calix[4]arene L-proline derivatives**			**Reference**
Concentration range [mM]	0.625-5.000					[3]

active site and conformation of the enzyme by creating electrostatic interaction and hydrogen bonding with acidic and basic amino acid residues in the structure of the enzyme have been observed [2]. In other work [4], the surfactants were added to the reaction of enantioselective hydrolysis of ketoprofen ester, catalyzed by lipase OF from *Candida rugosa* (CRL-OF) to obtain a chirally pure (*S*)-ketoprofen. Including the applied compounds, high catalytic parameters (conversion, *C*) were achieved in the reaction with the addition of Tween 80 (*C* = 46%). The functions of surfactants in the performed process could include the stabilization of emulsion, the lipase activator, and the enantioselectivity enhancer.

Lipase B from *Candida antarctica* (CALB)

Lipase B from *Candida antarctica* (CALB) is an enzyme commonly applied in catalyzing various reactions with pharmaceutical significance. The most important of these reactions include the enantioselective transesterification of drug racemic mixtures to obtain pure enantiomers, and the hydrolysis of triglycerides of fatty acids to free fatty acids and glycerol. CALB was applied to the kinetic resolution of (*R,S*)-flurbiprofen to obtain (*R*)--flurbiprofen with better pharmacological activity than (*S*)-flurbiprofen. Numerous studies on the application of surfactants in the reactions catalyzed by CALB have been described. Quilles *et al.* [5] studied the effect of surfactants (SDS, CTAB, and Triton X-100) in various concentrations (Table 2) (0.05, 0.1, 0.2%) on immobilized CALB activity. The results showed a negative influence of CTAB and SDS and a positive influence of Triton X-100 on CALB enzymatic activity. The authors suggested that the cationic surfactants were more optimal for enzymes with anionic characteristics. In turn, the neutral surfactant strongly affects the CALB activity of the positive form [5]. Moreover, the enzymatic activity could be slightly increased due to the dissociation of the formed bimolecular aggregates promoted by the surfactant. In turn, Zheng *et al.* [6] tested the effect of surfactant concentration on CALB activity and enantioselectivity. CALB catalyzed the enantioselective hydrolysis of *N*-(2-ethyl-6-methylphenyl) alanine to obtain chirally pure (*S*)-*N*-(2-ethyl-6-methylphenyl) alanine, an important precursor in herbicide synthesis. The surfactants – Tween 80, CTAB, and Bis(2-ethylhexyl)sodium sulfosuccinate (AOTNa) were added to the reaction mixture in different concentrations (Table 2). The relative activity of CALB (above 100%) has been achieved with all tested surfactants. However, the highest

Table 2. The concentrations of surfactants tested in the reactions catalyzed by CALB.

Surfactant	SDS	CTABr/CTAB	Triton X-100	Reference
Concentration range [%]		0.05–0.2		[5]
Surfactant	**Tween-80**	**CTAB**	**AOTNa**	**Reference**
Concentration range [mg/mL]		10–60		[6]

value (above 200%) was reached with the application of CTAB in the concentration of 30–60 mg/mL. On the other hand, the highest enantioselective ratio was obtained using Tween-80 as a surfactant – the *E* value was 60.1 in the concentration of 20 mg/mL. The authors suggested that the positive effect of Tween-80 could have resulted from the non-ionic character of the surfactant, hydrogen bonds, and hydrophobic interactions between Tween-80 and lipase. In other studies [7], the surfactants were received in the esterification of glucose catalyzed by CALB. The created glucose esters could be applied in the pharmaceutical and cosmetology industries (detergents, emollients). The structures of Tween 80, Triton X-100, and AOTNa are shown in Figure 5.

Lipase from *Burkholderia cepacia* (BCL)

The surfactants (AOT, CTAB, Tween-80, and Triton X-100) have been used to activate the lipase from *Burkholderia cepacia* (*Pseudomonas cepacia*) – BCL (PCL) [8]. BCL is, among others, one of the most widely applied enzymes to catalyze the reaction of biodiesel production. However, this lipase is also applied as a catalyst for obtaining

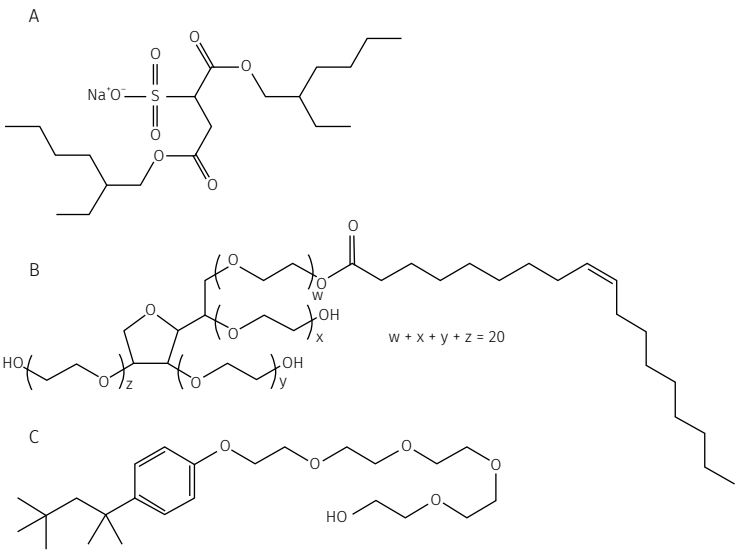

Figure 5. The structures of surfactants tested in reaction catalyzed by CALB: AOTNa (A), Tween 80 (B), Triton X-100 (C).

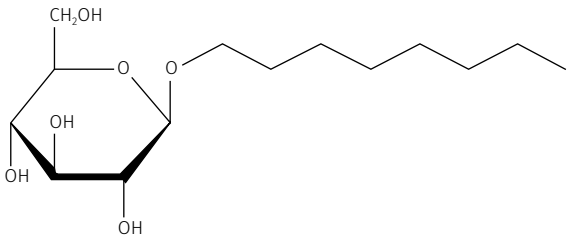

Figure 6. The structure of *n*-octyl-β-D-glucopyranoside (OG), tested in the reaction catalyzed by BCL.

optically pure enantiomers of drugs or building blocks [9–11]. Analogously to most lipases, BCL contains a lid in its structure. The activation of BCL by surfactants is based on facilitating the lid opening [8]. In addition, BCL was immobilized in magnetic cross-linked enzyme aggregates (mCLEAs) with hydroxyapatite-coated magnetic nanoparticles (HAP-coated MNPs). The concentration of surfactants was in the range of 0.1–2.0 mM. BCL activity, expressed as biodiesel yield was the highest with low surfactant concentration (0.1–0.5 mM) [8]. This fact could be caused by blocking the accessibility to the substrate by increasing the surfactant concentration. In other studies [12], the effect of *n*-octyl-β-D-glucopyranoside (OG) (Figure 6) on BCL enantioselective activity has been tested. OG has been added to the sample containing free BCL and the enzyme precipitated and rinsed with organic solvents (EPROS). The study of the enantioselective activity of BCL was performed in the kinetic resolution of (*R*,*S*)-1-phenylethanol. After 6 h of reaction, the high activity of lipase was achieved in BCL-EPROS with the addition of OG, compared with free BCL and BCL-EPROS without surfactant addition. The conversion (*C*) was 46%, which indicates a significant effect of surfactant on the lipase enantioselective activity.

Lipase from *Thermomyces lanuginosus* (TLL)

In performing the biocatalysis research, the selection of enzymes with high stability is required. Lipase from *Thermomyces lanuginosus* (TLL) belongs, among others, to the most thermally stable lipases [13]. Additionally, TLL shows high stability in basic pH. Devendran *et al.* [14] applied TLL in the kinetic resolution of (*R*,*S*)-1-(2-furyl)-ethanol to obtain the optically pure (*S*)-1-(2-furyl)-ethanol, an important chiral building block in the pharmaceutical synthesis of polyketide antibiotics. The effect of surfactants on TLL activity has been studied by Mesa *et al.* [13]. The enzyme activity assay has been performed by hydrolyzing *p*-nitrophenyl butyrate (*p*-NPB) with the introduction of Triton X-100 in a 48-hour of incubation sample. The results showed that the presence of Triton X-100 had a hyperactivating effect on TLL activity in pH 5, without maintenance after 48 h. The authors suggested the surfactant addition causes structural changes at 0 h and 48 h, especially at non-neutral pHs. In a different study, Sibalić *et al.* [15] tested the activity of TLL with the application of various surfactants as emulsifiers: Gum Arabic, polyoxyethylene glycol (PEG) 4000, 6000, Triton X-45, SDS, CTAB, Tween 20, Tween 40, Tween 80, and Span 80 (Figure 7).

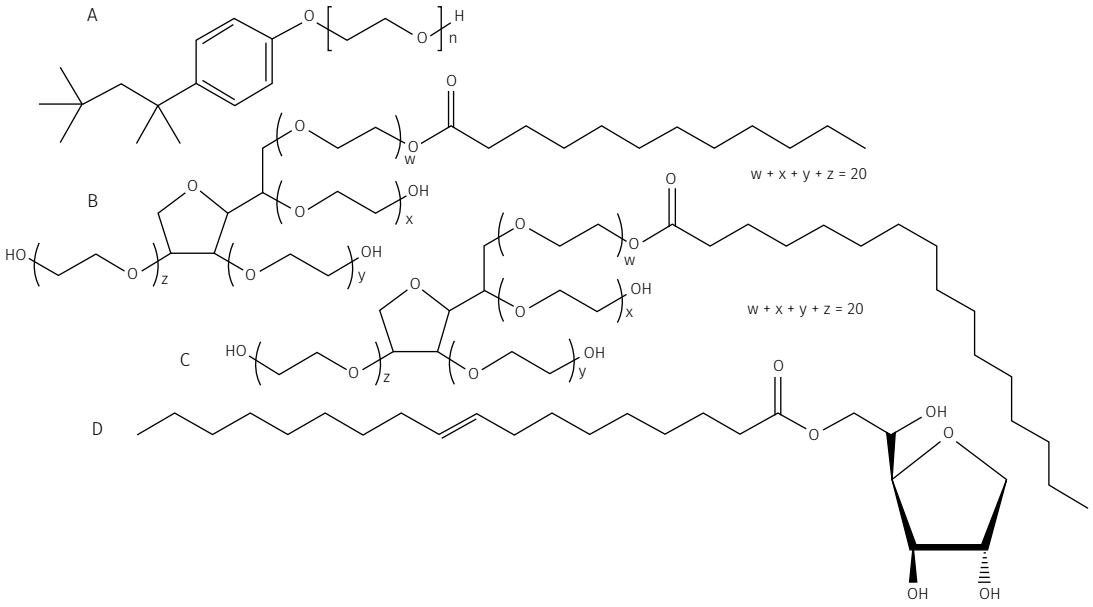

Figure 7. The structures of selected surfactants studied in reaction catalyzed by TLL lipase: Triton X-45 (A), Tween 20 (B), Tween 40 (C), Span 80 (D).

Table 3. The concentrations of surfactants tested in reactions catalyzed by TLL.

Surfactant	Triton X–100		Reference
Concentration [M]	0.001		[13]
Surfactant	**PEG 4000 and 6000, Triton X–45, SDS, CTAB, Span 80, Tween 20, 40, 80**	**Arabic Gum**	**Reference**
Concentration range [%]	0.05–0.2	0.3–0.5	[15]

The highest activity of TLL has been achieved by applying Gum Arabic, whereas the application of other surfactants deactivated the lipase. The concentrations of surfactants in reactions catalyzed by TLL are juxtaposed in Table 3.

Lipase from *Pseudomonas fluorescens* (PFL)

The effect of surfactants on catalytic properties of lipase from *Pseudomonas fluorescens* (PFL) has been studied by Rios *et al.* [16]. PFL was applied as the catalyst for achieving fatty acid amides (FAA) from Brazil nut oil (BNO) [17]. The obtained FAA was characterized by analgesic, antimicrobial, antituberculosis, and anti-inflammatory activities. Degórska *et al.* [18] carried out the enzymatic resolution of 3-hydroxy-3-phenylpropanonitrile (3H3P) catalyzed by PFL. One of the products of the reaction was pure (*S*)-3-hydroxy-3-phenylpropanonitrile ((*S*)-3H3P), an important intermediate in fluoxetine synthesis. It should be mentioned that PFL is known as the enzyme with characteristic features to form bimolecular aggregates, causing the blockage of the enzyme's active site of paired lipase molecules [16, 19]. Rios *et al.* [16] analyzed the effect of surfactants: anionic (SDS), cationic (CTAB), and non-ionic (Triton X-100) and their results showed that the high concentration of SDS and CTAB (0.05%, 0.1%) decreased the PFL activity. On the other hand, the application of Triton X-100 in the concentration of 0.1% increased the PFL activity. The authors attributed this tendency to the stabilization of the lipase open form.

Lipase from *Pseudomonas aeruginosa* (PAL)

The surfactants have also been used in reactions catalyzed by lipase from *Pseudomonas aeruginosa* (PAL). The application of PAL in the pharmaceutical area is poorly described in the literature. It should be mentioned that PAL is characterized by high catalytic parameters. Despite the possession of a lid, the interfacial activation of PAL has not been observed [20]. Shoja *et al.* [21] studied the effect of non-ionic surfactants, Tween 20 and Tween 80, on PAL activity at various temperatures and pHs. The results showed the positive effect of Tween 20 and Tween 80 on lipase activity at 30–60 °C and alkaline pH 8–9. The authors suggested that the non-ionic surfactants activated the lipase without protein destabilization. Therefore, PAL used in detergent formulations should maintain its catalytic activity in the presence of harsh conditions [21].

Lipase from *Pseudomonas stutzeri* (PSL)

Describing another *Pseudomonas sp.* lipase, it should be mentioned that the lipase from *Pseudomonas stutzeri* (PSL) is applied in the pharmaceutical industry as the catalyst of reaction in obtaining the chiral building blocks of drug synthesis [22]. The main example is the application of isolated PSL ZS04 in the enantioselective esterification of (*R*)-1-(4-methoxyphenyl)-ethanol (MOPE). In the performed study [22], the effect of surfactants has been studied. The results showed the activation effect by using Tween 20, Tween 80, Triton X-100, and Gum Arabic. In turn, SDS caused inhibition of PSL activity. In other studies [23], the authors observed inhibition of PSL lipolytic activity in the presence of SDS, CTAB, and sodium lauryl sulfate (IPC-SDS), caused by the effect of ionic surfactants on the inactivation of the lipase globular part. On the other hand, non-ionic detergents, due to the increase in the conformation flexibility of the lipase active site, resulted in a slight positive effect on lipase activity.

Lipase from *Aspergillus oryzae* (AOL)

Lipase from *Aspergillus oryzae* (AOL) is an enzyme from *Aspergillus sp.* produced by genetic recombination. The application of AOL in reactions with pharmaceutical significance has increased in recent years. Li *et al.* [24] described the synthesis intermediate of brivaracetam, an important antiepileptic drug. The reaction was catalyzed by lipase M16 AOL WZ007. In other studies [25], AOL WZ007 has been used to catalyze the reaction to achieve pure (*R*)-ethyl 2-bromoisovalerate, the pharmaceutical intermediate in fluvalinate preparation. Among other lipases (CALB, BCL), AOL was also applied in the kinetic resolution of (*R,S*)-1-phenylethanol to achieve chirally

pure (*R*)-enantiomer [26]. The effect of surfactants on AOL activity has been studied by Ma *et al.* [27]. Lipase was immobilized onto copper phosphate trihydrate ($Cu_3(PO_4)_2{\cdot}3H_2O$) via biomineralization and the synthesis of lipase-($Cu_3(PO_4)_2{\cdot}3H_2O$) hybrid microflowers (Lip-MFs) was optimized. The surfactants (CTAB, Triton X-100, and Tween 80) were added to create the precipitate. The lipase activity was measured in the hydrolysis of *p*NPP. The achieved results showed high activity of CTAB-Lip-MFs, compared with free AOL and the Lip-MFs, whereas the activity of Triton-Lip-MFs and Tween-Lip-MFs showed higher activity than Lip-MFs, however, lower than free lipase. The authors explained that the high catalytic parameter of CTAB-Lip-MFs is caused by the interfacial action of CTAB, following lipase open conformation. Moreover, this surfactant could protect against the aggregation of lipase molecules through appropriate dispersion in the reaction mixture.

Lipase from *Aspergillus niger* (ANL)

Lipase from *Aspergillus niger* (ANL) is gained from *Aspergillus sp.* mainly by genetic recombination and fermentation. However, ANL is also a commercially available lipase. As the commonly applied enzyme in biocatalysis, ANL has been used in reactions to obtain the building block in the synthesis of macrolides antibiotics [28]. According to recent studies [29], free ANL is characterized by low enzymatic activity. However, the immobilization significantly increased the catalytic parameters. Bhowal *et al.* [30] tested the effect of surfactants on ANL activity. The increased concentration of non-ionic surfactant (Tween 80, Span 80) for all studied concentrations of ionic surfactant (CTAB) resulted in increasing lipase recovery activity [30].

Lipase from *Yarrowia lipolytica* (YLL)

Lipase from *Yarrowia lipolytica* (YLL) is an enzyme commonly used in the pharmaceutical industry. YLL shows high activity in the kinetic resolution of (*R*,*S*)-2-bromophenyl acetic acid ester racemates and (*R*)-enantioselectivity for the resolution of the ethyl ketoprofen ester racemate [31]. However, literature data show that YLL has low enantioselectivity in racemic ibuprofen resolution. [31]. In other studies, YLL was used in the pathway of terpenoid synthesis [32]. According to Aloulou *et al.* [33], YLL Lip2p, due to its maximal activity in low pH and resistance to bile salts, seems to be the optimal lipase for enzyme replacement therapy. In this study, the effect of surfactants on YLL Lip2p activity has been studied. The assay was performed in the presence of non-ionic (Triton X-100, β-octyl glucoside), anionic (SDS, sodium taurodeoxycholate – NaTDC), cationic (benzalkonium chloride, tetradecyl trimethylammonium bromide – TTAB) (Figure 8) and zwitterionic (Zwittergent 3-12) surfactants. The obtained results showed the activation of lipase in low concentrations of surfactants (for SDS – in CMC concentrations below 0.125 mM). The YLL activity was abolished or drastically decreased above the CMC of Triton X-100, and a lack of activity in cationic and zwitterionic detergents was observed.

Lipase from *Rhizomucor miehei* (RML)

Lipase from *Rhizomucor miehei* (RML) is an enzyme with a high catalytic activity. However, RML tends to form bimolecular aggregates with reduced activity [34]. This enzyme is commercially available and was applied in the production of low-calorie structural lipid (SL), used in the pharmaceutical industry [35]. In turn, de Oliveira *et al.* [34] studied the effect of surfactants (SDS, Triton X-100, CTAB) on RML catalytic properties.

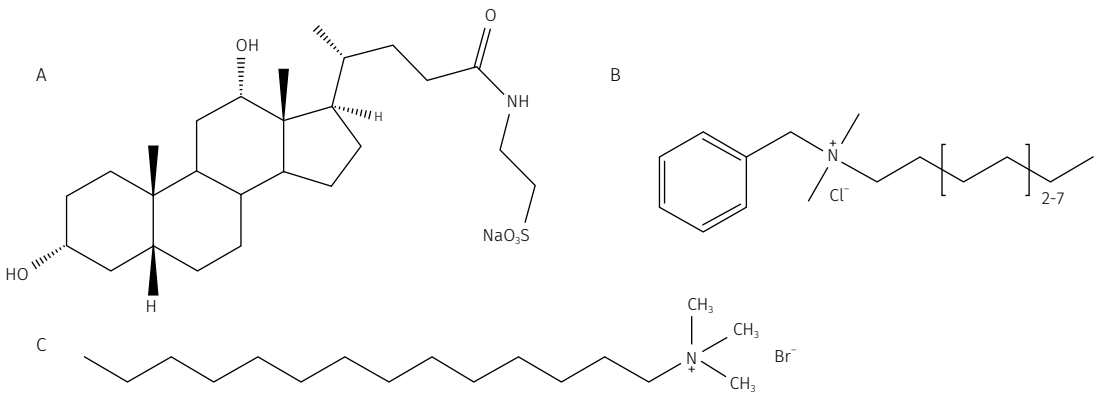

Figure 8. Structures of selected surfactants tested in the reaction catalyzed by YLL lipase: NaTDC (A), benzalkonium chloride (B), and TTAB (C).

Table 4. The juxtaposition of the effects of the most commonly applied surfactants on various lipase activities. "↑" means positive effect (including hyperactivation), "↓" negative effect, "↑/↓" both positive and negative effect "-"No data. "*" – the decrease in activity after exceeding the CMC of surfactants.

Lipase \ Surfactant	SDS	CTAB/CTABr	Tween 20/40/80	Triton X-45/100/114	Cyclic	Gum Arabic	References
CRL	↑	↓	↑ (80)	-	↑	-	[2-4]
CALB	↓	↑↓	↑	↑ (100)	-	-	[5-7]
BCL	-	↑	↑ (80)	↑ (100)	-	-	[8, 12]
TLL	↓	↓	↓	↑ (100); ↓ (45)	-	↑	[13, 15]
PFL	↓	↓	-	↑ (100)	-	-	[16]
PAL	-	-	↑ (20; 80)	-	-	-	[21]
PSL	↓	↓	↑ (20; 80)	↑ (100)	-	↑	[22, 23]
AOL	-	↑	↑ (80)	↑ (100)	-	-	[27]
ANL	-	↑	↑	-	-	-	[30]
YLL	↑	-	-	↓ (100)	-	-	[33]
RML	↑	↓	-	↑*	-	-	[34]
FOL	↓	-	-	↑	-	-	[37]

The assay of enzyme activity was performed by hydrolyzing *p*NPB. Analyzing the results, RML showed high enzymatic activity in the presence of Triton X-100. However, after exceeding the CMC, the activity declined. This fact could be caused by binding the lipase active site by the excess surfactant above CMC. In turn, the application of a low concentration of CTAB caused a slight increase in lipase activity, whereas full inhibition of lipase took place after increasing the surfactant amount. On the other hand, the presence of SDS increased RML activity. The authors also suggested that the binding of SDS to the protein surface makes the structure more flexible. This phenomenon allows for the accommodation of more substrate molecules and, thus, an increase in lipase activity.

Lipase from *Fusarium oxysporum* (FOL)

Lipase from *Fusarium oxysporum* (FOL) is an extracellular lipase with specificity for saturated acids. FOL has been applied in the synthesis of 2-hydroxy-2-(ethoxyphenylphosphinyl)acetic acid and its ester [36]. This compound belongs to chiral hydroxyphosphonates, which show antibacterial, antiviral, and antitumor activities. Dos Prazeres *et al.* [37] tested the effect of various surfactants on FOL activity. The reaction was performed in the presence of surfactants (Tween, Triton-X) and other commercial detergents. Based on the obtained relative activity, the lipase showed the highest activity with the application of non-ionic surfactants – Triton X-100 and Triton X-114. In turn, in the presence of SDS, a strong inhibition of lipase activity occurred. According to the author's suggestion, the SDS, due to the change in lipase conformation, caused the partial reversible unfolding of the enzyme structure [37]. On the other hand, the application of commercial detergents resulted in lower enzymatic activity than reactions using surfactants (Tween, Triton-X).

Summary

Based on the cited references, surfactants demonstrate a substantial impact on a range of lipase activities. The juxtaposition of these dependencies is presented in Table 4. The frequency of used surfact ants is presented in Figure 9.

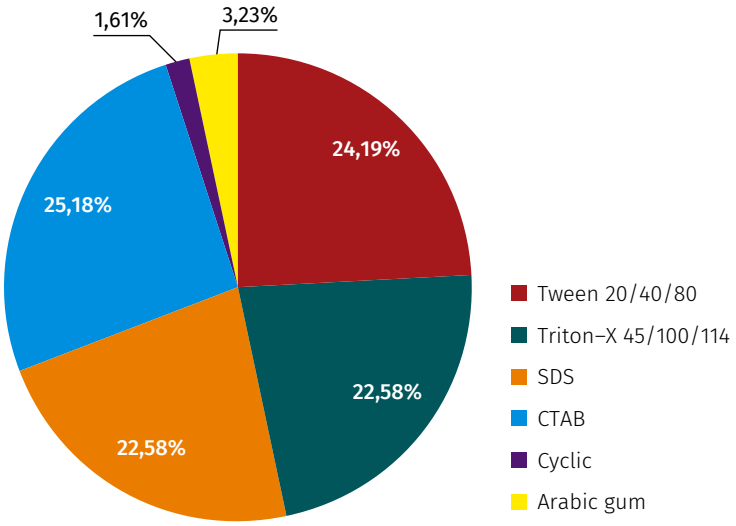

Figure 9. The graph shows the most frequently used surfactants. The comparison assumes the ratio of the amount of literature data describing used surfactants to the total number of all surfactants (many articles describe the use of more than one surfactant).

Conclusions

Surfactants are a wide group of compounds applied in the medical and pharmaceutical industries. The differentiation of the chemical structures of these compounds – cationic, anionic, amphoteric (zwitterionic) and non-ionic, ensures their diverse behavior in the reaction systems. Therefore, surfactants find wide application in biocatalysis reactions. The literature review concerning the presence of surfactants in reactions catalyzed by lipases showed the significant effect of tested detergents on lipase activity. In most cases, the non-ionic surfactants (Tween, Triton-X) showed a beneficial effect on enzyme activity. On the other hand, an inhibition effect with the application of ionic (mainly anionic) surfactants has been observed. The significance of detergents belongs to the novel trend of biocatalysis studies and requires further research.

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