

BIFUNCTIONAL SURFACTANTS - THEIR POTENTIAL APPLICATION AS PESTICIDES

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A b s t r a c t. Recently, a new class of surfactants that combines two contradictory functions has appeared. They can be used as effective antioxidants protecting model and biological objects against peroxidation and its negative consequences, thanks to the antioxidant functional group incorporated into the hydrophilic part of the molecule. On the other hand, they may be applied as common pesticides thanks to their intensive interaction with biological membranes that leads to destruction and death of the unwanted microorganisms. Both applications depend on the concentration of the bifunctional surfactants (BS) used. The work contains results of the studies on a possible pesticide application of the bifunctional surfactants. Planar lipid membranes (BLM) were used as model membranes.

K e y w o r d s: bifunctional surfactants, planar lipid membrane, pesticides

INTRODUCTION

The invention of the so-called black lipid membrane in 1962 (BLM) [25] gave scientists a very convenient and powerful tool for studying various properties of biological membranes that the BLMs were imitating. BLM have also been used, among others, to study their interaction with various substances, including those used in agriculture. Such approach enables us to understand the mechanism of the pesticidal activity of some groups of substances, especially those of amphiphilic character. The quaternary ammonium salts studied here belong to such a group of substances and, moreover, they have an antioxidant functional group incorporated into their polar part. Thus, they constitute a new class of bifunctional surfactants (BS) whose antioxidant efficiency was found to be quite

good [20] and in some cases even better than that of standard or biological antioxidants [14, 15, 18, 19].

The aim of the present work was to estimate the pesticidal efficiency of BS and to determine if such an efficiency qualifies them for agricultural application.

The method applied was to observe the stability of model membranes in the presence of increasing concentration of the BS studied. The parameter observed was the critical concentration of a compound (CC), i.e., the concentration that caused a complete destruction of a BLM in less than 3 min. The method proved to give satisfactory results in the sense of predicting whether a particular compound can be used as biologically active [31].

MATERIALS AND METHODS

All the antioxidants studied, presented in Fig. 1, were synthesized in the Institute of Organic and Polymer Technology, Technical University, Wrocław, Poland. The methods of their synthesis are described in [14, 19]. Purity of the synthesized compounds was checked by ¹H-NMR spectra.

Planar lipid membranes (BLM) were formed from a solution of 1.5% (w/v) azolectin in n-butanol:n-decane (1:1, v/v) on a 1.7 mm hole in a partition of a two-compartment chamber filled with a 0.9% NaCl solution. Appropriate volumes of antioxidant solutions were added

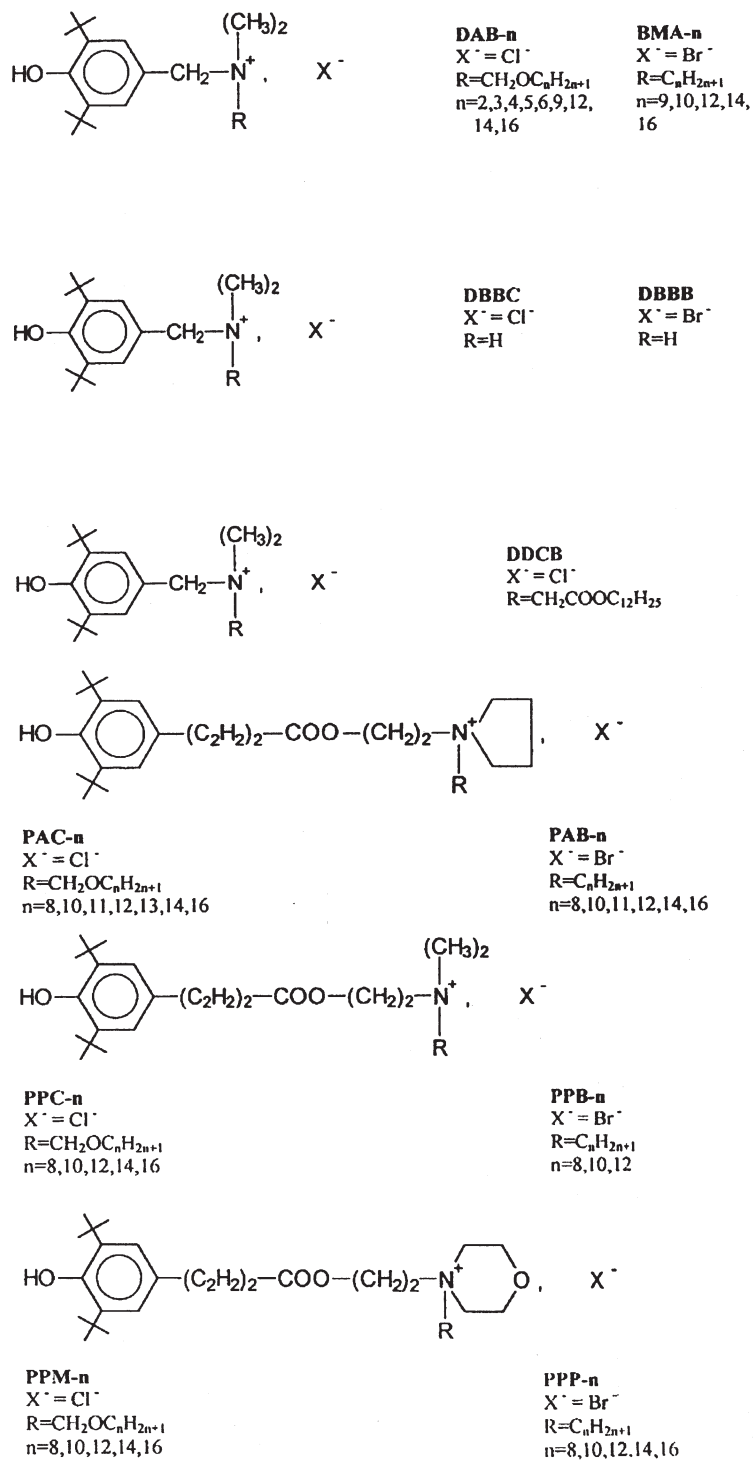


Fig. 1. General formulas of the bifunctional surfactants studied.

directly into the chamber until their concentration reached the value that caused a breakdown of the membranes in the time period shorter than 3 min. Such concentrations are farther on referred to as critical concentrations (CC). The time was chosen arbitrarily. It was long enough to exclude the possibility of accidental breakage of the model membrane during the procedure of adding a compound solution to the chamber. The BLMs were continuously monitored by observing the current with a measurement system consisting of a Keithley 617 Proframmable Electrometer and a standard voltmeter controlling the d. c. voltage (20 mV) applied to the membrane by means of calomel electrodes immersed in the bath solution. The temperature was ~ 22 °C.

RESULTS AND DISCUSSION

The results of the experiments are collected in Table 1. Each row in Table 1 presents values of the critical concentrations (CC) found for the compounds of the same polar part. It facilitates comparison of the efficiency levels of the compounds in the destruction of the planar model membranes.

It is known that chemical characteristics of biologically active compound as well as environmental and weather conditions are responsible for the compound activity [16,22,24,37]. Polar head stereochemistry, including net charge and its distribution, the number and length of alkyl chain(s), presence of various spacer groups that can change hydrophobicity of a compound, are the most important chemical features of amphiphilic compounds that decide on their usefulness as pesticides. Moreover, in the case of the compounds studied, all those factors determined their efficiency in destroying model membranes. Namely, it was found out that the critical concentrations of bifunctional surfactants depended on the length of their hydrophobic parts which is the hydrocarbon (alkyl) chain or alkyl chain plus a spacer group (-CH₂O- or -CH₂COO-). The two spacer groups, as it was found in the previous experiments, modify the potential biological effectiveness of the compounds studied in otherwise. The oxymethylene group was found to increase hydrophobicity of a molecule almost to the same degree as two methylene groups [32]. This enabled the oxymethylene group

Table 1. Values of the critical concentrations (CC) of the bifunctional surfactants studied

Chlorides		Bromides		Chlorides		Bromides	
compound code name	critical concentration (μM)	compound code name	critical concentration (μM)	compound code name	critical concentration (μM)	compound code name	critical concentration (μM)
DAB-2	720			AAC-8	240	AAB-8	99
DAB-3	540			AAC-10	200	AAB-10	83
DAB-4	440			AAC-12	83	AAB-12	75
DAB-5	400			AAC-14	41		
DAB-6	400			AAC-16	16		
DAB-9	240	BMA-9	83				
		BMA-10	78	PAC-8	160	PAB-8	41
DAB-12	140	BMA-12	57	PAC-10	120	PAB-10	33
DAB-14	120	BMA-14	49	PAC-11	140	PAB-11	41
DAB-16	83	BMA-16	41	PAC-12	120	PAB-12	16
				PAC-13	110		
PPM-8	830	PPP-8	640	PAC-14	280	PAB-14	16
PPM-10	880	PPP-10	560	PAC-16	400	PAB-16	66
PPM-12	720	PPP-12	560				
PPM-14	560	PPP-14	520	DBBC	400	DBBB	200
PPM-16	520	PPP-16	480	DDCB	160		

Standard deviation did not exceed 10%.

containing compound to incorporate deeper into the lipid bilayer, thus enhancing the interaction with it. Contrary to the above, the CH_2COO group weakened that interaction due to the increased polarity of the compound resulting from the presence of a carbonyl group that contains a double bond between the carbon and oxygen atoms. This, in turn, diminished the possibility of the surfactant to incorporate into the lipid bilayer as deeply as a similar surfactant devoid of such a spacer group [28,32]. Additionally, different stereochemistry of both oxymethylene and alkoxycarbonylmethylene groups resulting in different stereochemistry of the whole molecule may lead to the differences observed in the interaction of the compounds with model membranes. The same remains valid for biological systems.

Generally speaking, the CC values inside a homologous series decreased with an increased hydrophobic part. However, some exceptions from this rule were observed. The maxima of the interaction were found while studying two homologous series of pyrrolidinium chlorides (PAC-n) and bromides (PAB-n). They appeared in the vicinity of $\text{C}_{12}\text{H}_{25}$ alkyl chain. A decreased biological efficacy of the compounds with longer hydrophobic parts than the optimal ones, has long been known [1,2,7-12,28-30,41,43]. There exist various theories explaining the phenomenon known as the cut-off phenomenon [1,2,9,39]. One of them postulates that an interdigitation effect is responsible. Interdigitation takes place when intruder molecules such as alcohols, drugs, anesthetics, some amphiphilic compounds and others, incorporate into the lipid bilayer formed of lipids with synthetic or biological origin [2,17,21,26,38,44]. The result is the bilayer state in which the terminal methylene groups of alkyl molecule chains of one monolayer can penetrate into the opposite monolayer, thus stabilizing the membrane. This may be the reason for the observed maxima in the efficacies of the two BS series.

The results summarized in Table 1, point out to the important role polar heads of the studied compounds play in their interaction

with BLMs. It seems that well developed polar parts of most of the bifunctional surfactants are not a limiting factor decreasing the intensity of the interaction with model membranes. On the contrary, pyrrolidinium (PAC-n and PAB-n) and alkylammonium salts (AAC-n and AAB-n) were found to be more effective than benzylammonium salts (DAB-n, BMA-n, DBBC, DBBB and DDCB). This conclusion is evident in the case of all chlorides. No such big differences were found for bromides. It also seems that the presence of an interspace group of dipolar character ($-\text{C}_2\text{H}_4\text{COOC}_2\text{H}_4-$) and the enhanced overall polarity of those compounds, in comparison to that of benzylammonium salts, is the reason for a more intensive interaction with model membranes. This interaction was the weakest in the case of morpholinium (PPM-n) and piperidinium (PPP-n) salts, and this may be caused by the presence of morpholinium and piperidinium rings which do not promote biological activity of the compounds; as was found in the earlier studies on some morpholinium and piperidinium salts synthesized for potential application as pesticides [31].

It was found that a model membrane that modifies possibilities of the studied compounds strongly depended on the kind of counterion of bifunctional surfactants. All bromides significantly stronger influenced BLMs than the corresponding chlorides [15,20,33-35]. Apparently, it may be attributed to different mobilities and radii of the hydrated chloride and bromide ions [11], which resulted in a quantitatively differentiated interaction of those anions with model membrane and subsequent differences in the modification of surface potential of those membranes. Moreover, it was shown that different halides bind their first hydration shell more or less tightly with the resulting effect on the solute solubility [3-6,40]. Both chloride and bromide anions are classified as the so-called chaotropes, i.e., water disordering ions that facilitate cationic surfactant incorporation into the lipid membrane. Moreover, they increase surfactant solubility in aqueous media thus changing the partition coefficient. A more chaotropic

of the halide ions is bromide. This may explain why the efficiency of bromide compounds to destruct BLMs is decidedly higher than those of chloride compounds. It is worth mentioning that this happens even though chloride salts have longer hydrophobic parts than the corresponding bromides (e.g., PAC-12 and PAB-12) due to the oxymethylene spacer group. They could be expected to interact with the BLM stronger than bromides. Nothing like that was observed.

The obtained values of the critical concentrations were compared with those compounds which biological activities were also tested *in vitro*. As it was already mentioned, the comparison of CC values with the results of biological tests gave satisfactory conclusions about the possible usefulness of the compounds. The applied evaluation method was as follows: The compounds were divided into three classes. Those causing 100% inhibition of microorganisms at the concentrations not higher than $1 \mu\text{g dm}^{-3}$ or destroying BLMs at the concentration not higher than $10 \mu\text{M}$ were defined as highly effective pesticides. Those compounds which caused the same inhibition at the concentrations $<10 \mu\text{g dm}^{-3}$ and $<100 \mu\text{g dm}^{-3}$, respectively were classified as fairly effective and slightly effective. The corresponding values of the CC were $<50 \mu\text{M}$ and $<100 \mu\text{M}$, respectively [31]. The bifunctional surfactants studied were classified according to this arbitrary estimation method for the potential biological activity.

CONCLUSIONS

The classification method used showed that most of the bifunctional chlorides studied can be treated as poor pesticides. The longer alkyl chain AAC-n compounds (n=12, 14 and 16) are exceptions. The obtained CC values classified those compounds as effective potential pesticides and biological tests confirmed that. They were found to be good inhibitors of *S. Cerevisiae* [42]. As it has been already said, chloride compounds were effectively used as antioxidants. Their worse pesticide efficiency is in such a case an advantage. When applied at pro-

per concentrations, their antioxidative properties can be fully useful and at the same time they do not cause damage to the model and/or biological membranes they protect. On the other hand, bromides, with the exclusion of piperidinium and one of the benzylammonium salts (PPP-n and DBBB), exhibited significantly better efficiencies in destroying planar lipid membranes than chlorides, and can thus be used in both roles. Subject to demand, they can serve as efficient antioxidants or as effective pesticides.

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