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New piperidinium surfactants containing *N*-ethylcarbamate fragment: aggregation properties and antimicrobial activity

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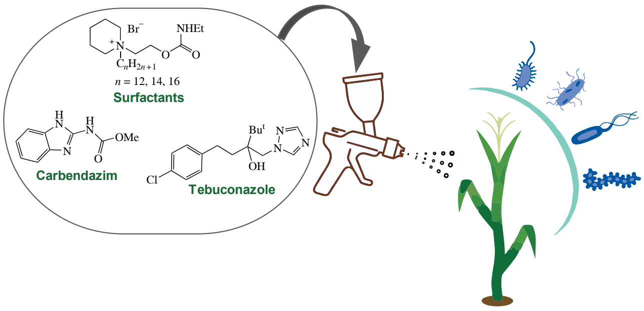
A homological series of novel piperidinium surfactants containing *N*-ethylcarbamate fragment and dodecyl, tetradecyl, or hexadecyl substituents was synthesized. Quantitative parameters describing their surface activity and aggregation behavior in water were determined using tensiometry and spectrophotometry. These cationic surfactants exhibit significant antimicrobial activity against several pathogenic plant strains, particularly bacteria, which increases with the transition from dodecyl to hexadecyl derivatives.



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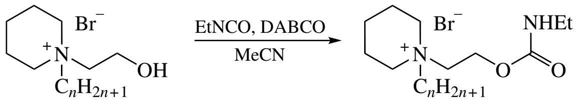
The wide practical use of cationic surfactants in industry, agriculture, and medicine has led to a significant number of studies aimed at developing new effective and safe compounds of this family.[1–4] A systematic investigation of the aggregation behavior and functional activity of such surfactants can provide insight into key factors responsible for their practical application.[5,6] In addition, important tasks are the examination of concentration limits of their functional activity and identifying the leading compounds. Previous[7–9] studies on acyclic cationic surfactants containing carbamate fragments showed that they differed from their non-functionalized trialkylammonium analogs in terms of a lower critical micelle concentration (CMC) and Krafft temperature. Additionally, they had high solubilization, wetting, and antimicrobial properties, as well as lower toxicity. In this work, we proceed with the search for new carbamate-containing surfactants, which can be used as effective solubilizers, adjuvants, and antimicrobial agents. To achieve this goal, a series of piperidinium surfactants functionalized with an *N*-ethylcarbamate fragment were synthesized and investigated. Homologs with dodecyl, tetradecyl, and hexadecyl substituents (salts **1**, **2**, and **3**, respectively) were obtained by the reaction of the corresponding 1-alkyl-1-(2-hydroxyethyl)piperidinium bromides with ethyl isocyanate using diazabicyclooctane as a catalyst (Scheme 1, *cf.* ref. 10). This series of surfactants was chosen to make a compromise between their solubility,



1 $n = 12$
2 $n = 14$
3 $n = 16$

Scheme 1

aggregation activity, and functional efficacy. The structure of the compounds was confirmed using IR- and NMR-spectroscopy, mass spectrometry, and elemental analysis (for details, see Online Supplementary Materials and Figures S1–S9 therein).

In this study, a series of quantitative parameters were obtained to describe the behavior of piperidinium surfactants with the *N*-ethylcarbamate fragment in aqueous solutions. These parameters include their surface activity, micellization, and solubilization properties (the methods and instrumentation are detailed in the Online Supplementary Materials). The CMC values of the surfactants were determined based on the surface tension isotherms, which were obtained using the Du Noüy ring method, with an accuracy of ±0.1 mN m^{-1} (Figure 1). As shown in Table 1, the CMC values of the synthesized surfactants are significantly lower compared to their trimethylammonium analogs, including cetyltrimethylammonium bromide CTAB. These values correlate with the length of the alkyl substituent (n) in accordance with the equation $\log(\text{CMC}) = 1.52–0.3n$ ($R^2 = 0.9998$) (Figure S10).

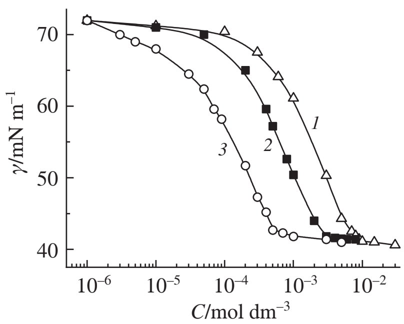
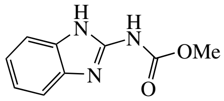


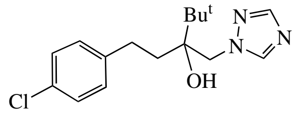
Figure 1 Surface tension isotherms of aqueous solutions of piperidinium surfactants **1**–**3** containing the *N*-ethylcarbamate fragment, 25 °C.

Table 1 Aggregation properties of the homologous series of piperidinium surfactants containing the *N*-ethylcarbamate fragment at 25 °C.

Surfactant	CMC × 10^3/mol dm^{-3}		γ_{CMC}/ mN m^{-1}	θ/deg	S^a	$N_{agg}{}^b$
	Tensiometry	Orange OT				
1	7.0	8.0	42.5	76	0.008	127
2	2.5	2.0	42.0	74	0.015	68
3	0.5	0.4	39.4	72	0.021	42
CTAB	0.9	1.0	41.1	77	0.015	–

[a] *S* is the solubilization capacity. [b] Surfactant aggregation numbers calculated at a concentration of a 3-fold CMC.


Carbendazim


Tebuconazole

At the CMC point, the values of surface tension at the water/air interface (γ_{CMC}) and the contact angles (θ) of the cationic surfactants synthesized in this study were determined (see Table 1). Measurements were taken using a KRUSS 100 automatic tensiometer. The wetting force was determined by immersing a solid sample (Parafilm M type), which mimics the waxy cuticle of plant leaves, into a micellar solution with a known surface tension. This method was used to evaluate how effectively micellar solutions can improve the distribution of agrochemicals on plant surfaces. The results showed that a transition from dodecyl to hexadecyl homolog leads to a decrease in surface tension and a reduction in the contact angle (see Table 1). These changes indicate an increase in the surface activity of the surfactants. The contact angles for the investigated compounds were significantly lower compared to the water control (103°). Low contact angle values correlate with the high adsorption activity of the tested surfactants at the solution/air interface. The ability to improve the wettability of hydrophobic surfaces and the significant solubilization effect of surfactants are important for their use as adjuvants in biomedical and agricultural formulations.

The solubilization properties of the synthesized surfactants were investigated using a spectrophotometric method with the dye Orange OT as a hydrophobic probe, following the described[8] procedure. Based on the obtained solubility isotherms of this dye (Figure S11), the solubilization capacity (*S*) and CMC values of the surfactants were determined. Additionally, the aggregation numbers (N_{agg}) were evaluated using the Schott approach.[11] Noteworthy, the CMC values obtained using this method are in good agreement with the data from tensiometry. The aggregation numbers determined at a concentration by three times higher than the CMC decreased as the length of the hydrocarbon chain increased (see Table 1). Compound **3** showed the best solubilization properties towards Orange OT among the tested surfactants. This surfactant was used to improve the solubility of two active ingredients, carbendazim and tebuconazole, which are the basis of several commercial fungicide products. Based on spectral data (Figures S12 and S13), it was found that the addition of a 0.1% wt. solution of salt **3** increased the solubility of tebuconazole in water by approximately fourfold and the solubility of carbendazim by 1.5 times. This, in turn, may enhance the effectiveness of these fungicides in practical applications.

The antimicrobial activity of synthesized surfactants **1–3** was tested against a range of pathogenic microorganisms that cause plant diseases and result in significant losses in agriculture.[12,13] The antibacterial and antifungal activities of the tested substances were evaluated using the double serial dilution method, following the described[14,15] protocols. The test cultures included strains of gram-positive bacteria such as *Clavibacter michiganensis* BKM Ac-1404 (*Cm*), *Bacillus subtilis* BKM B-12 (*Bs*), *Rathayibacter iranicus* BKM Ac-1602 (*Ri*) and *Curtobacterium flaccumfaciens* BKM Ac-1923 (*Cf*); gram-negative bacteria such as *Erwinia carotovora* spp. *Carotovora* (*Ec*), *Azotobacter vinelandii* BKM B-1273 (*Av*) and *Xanthomonas arboricola* S3 (*Xa*); and fungi such as *Colletotrichum cocodes* BKM F-4298 (*Cc*), *Alternaria alternat* BKM F-3047a (*Aa*), *Alternaria solani* BKM F-3048 (*As*), *Fusarium graminearum* FG-30 (*Fg*) and *Fusarium oxysporum* BKM F-137 (*Fo*). All of these microorganisms were obtained from the All-Russian Collection of Microorganisms. Antibiotic chloramphenicol and the fungicides carbendazim and tebuconazole were used as reference compounds. Based on the data presented in Table 2, synthesized surfactants **1–3** demonstrate significant antibacterial activity that increases with the length of the alkyl chain from dodecyl to hexadecyl. For compound **3**, the minimum inhibitory concentration (MIC) values range from 3 to 6 μg ml^{-1}, which is significantly lower than the MIC of the bactericide chloramphenicol. Note that surfactant **3** exhibits high activity not only against gram-positive bacteria but also against gram-negative bacteria. Gram-negative bacteria are typically more resistant to antibiotics due to the presence of an additional membrane in their cell structure, which inhibits antibiotic action.[16,17]

However, the tested surfactants did not demonstrate significant activity against phytopathogenic fungi and were less effective than the tebuconazole (Table S1). Some selectivity was observed for compounds **3** and **1** against *As* and *Fg* (MIC 62.5 μg ml^{-1}). Testing of the biological activity of carbendazim has revealed its selectivity against fungi belonging to the *Fusarium* genus. In the case of other tested strains, the effectiveness of the fungicide was lower compared to the studied cationic surfactants (see Table S1).

The results suggest that the combination of surfactant **3** and active fungicide ingredients, such as carbendazim or

Table 2 Antibacterial activity of the piperidinium surfactants containing an ethylcarbamate fragment against various phytopathogenic microorganisms.

Compound	Minimum inhibitory concentration (MIC)/μg ml^{-1}						
	Cm	*Bs*	*Ri*	*Cf*	*Ec*	*Av*	*Xa*
1	50.0 ± 4.2	50.0 ± 5.4	50.0 ± 5.4	25.0 ± 3.6	20.0 ± 1.8	25.0 ± 1.2	25.0 ± 1.5
2	6.25 ± 0.9	12.50 ± 0.3	6.25 ± 0.2	6.25 ± 0.8	12.50 ± 0.7	3.12 ± 0.2	6.25 ± 0.5
3	3.12 ± 0.2	3.12 ± 0.2	3.12 ± 0.1	3.12 ± 0.3	6.25 ± 0.4	3.12 ± 0.1	3.12 ± 0.2
Chloramphenicol	19.0 ± 1.8	312.50 ± 34.0	19.0 ± 2.4	19.0 ± 1.0	312.50 ± 27.0	156.25 ± 13.0	156.25 ± 10.2
	Minimum bactericidal concentration (MBC)/μg ml^{-1}						
1	100.0 ± 8.4	100.0 ± 10.8	100.0 ± 10.8	50.0 ± 7.2	100.0 ± 9.0	50.0 ± 2.4	50.0 ± 3.0
2	12.50 ± 1.8	25.0 ± 0.5	12.50 ± 0.4	6.25 ± 0.8	25.0 ± 1.4	6.25 ± 0.4	12.50 ± 0.9
3	6.25 ± 0.3	3.12 ± 0.2	6.25 ± 0.2	3.12 ± 0.3	12.50 ± 0.8	6.25 ± 0.2	6.25 ± 0.4
Chloramphenicol	39.0 ± 3.6	625.0 ± 68.0	39.0 ± 4.8	39.0 ± 2.0	625.0 ± 54.0	312.50 ± 26.0	312.50 ± 20.4

tebuconazole, can lead to the development of an antimicrobial composition effective against both bacteria and fungi. Based on the analysis of MIC values, two formulations were selected for further tests. The first formulation contained salt **3** and carbendazim at a weight ratio of 5 : 1. The second formulation consisted of salt **3** and tebuconazole at a weight ratio of 1 : 3. These compositions effectively inhibited the growth of both phytopathogenic fungi and bacteria (Table S2). Notably, the carbendazim-based formulation showed a synergistic effect against the bacteria *Cm* and *Ec*. The MIC value for the combined composition was two times lower than that of the individual surfactant. The growth of the fungus *Fg* was inhibited at a total concentration of **3**/carbendazim of 15 μg ml^{-1}, which is 3 times higher than the MIC value of the fungicide used. However, recalculating the MIC values, taking into account the carbendazim content in the formulation (20%), suggests that its activity is doubled in the presence of surfactant **3**. The combined composition based on tebuconazole inhibited the bacterial growth at a concentration twofold higher compared to the MIC of compound **3**. However, considering the content of the surfactant in the formulation, it can be noted that its effectiveness slightly increases in the presence of the fungicide. The fungistatic activity of the tested formulation against *Aa* was by 5 times higher compared to the control preparations. In contrast, a pronounced synergistic effect was observed when fungi *Fg* were treated with the **3**/tebuconazole composition. In this case, the MIC and MFC values were an order of magnitude lower than those for tebuconazole (see Table S2).

It should be noted that the addition of carbendazim or tebuconazole to an aqueous solution of surfactant **3** did not change the surface activity of this surfactant: the contact angle and surface tension remained at 72.6° and 39.5 mN m^{-1}, respectively.

To conclude, the use of piperidinium surfactants containing a carbamate fragment in fungicidal formulations demonstrates a significant enhancement in their functionality compared to individual fungicide. Surfactants play an important role as adjuvants, increasing the solubility of active ingredients and improving the wetting of plant surfaces. It was shown that combining cationic surfactants with active fungicide ingredients provides effective tools against both bacterial infections and bacterial plant diseases. This is especially important in the light of the increasing resistance of pathogens to traditional methods of control.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.71267/mencom.7553.

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