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SYNTHESIS AND STUDY OF ANTIMICROBIAL PROPERTIES OF SURFACTANTS ON THE BASIS OF COTTON-SEED OIL, METHYLDİETHANOLAMİNE AND ORTHOPHOSPHORIC ACID

Abstract. Methyldiethanolamine esters have been synthesized on the basis of cotton-seed oil triglycerides and phosphated by orthophosphoric acid. The obtained products were identified by method of IR- spectroscopy. The surface activity of the synthesized substances was determined by tensiometric method. Based on these studies, the maximum adsorption and the minimum area of the molecule at the water /air interface, the surface pressure, the standard free Gibbs energies of adsorption and micellization have been calculated. Specific electroconductivity of the aqueous solutions of the surfactants were measured by electroconductometric method. Strong bactericide properties of the obtained surfactants were revelaed.

Key words: cotton-seed oil triglycerides, methyldiethanolamine, phosphate derivative of methyldiethanolamine, ester, surfactant, electroconductivity, micellization, bactericide

INTRODUCTION

Surfactants are widely used in various spheres of national economy [1,2]. Among them, the reagents obtained from ecologically -safe, and reproducible raw materials attract a special attention [3-5]. The present paper is devoted to synthesis of new representatives of such surfactants and a study of their antimicrobial properties.

EXPERIMENTAL

Cotton-seed oil is of a local production. Among residues of fatty acids contained in this oil triglycerides, of saturated ones, miristic acid (C_{14}) constitutes 0.3-0.5%, palmitic acid (C_{16}) - 20.0-22.0%, stearic acid (C_{18}) – 2.0%, arachinic acid (C_{20}) – 0.1-0.6% of unsaturated fatty acids, the content of residues of oleic acid (C_{18}) is 30.5-35.2% and that of linoleic acid (C_{18}) is 41.7-44.0%.

Methyldiethanolamine (MDEA) was the product of Russian Federation, molar mass-119.164 $g \cdot mol^{-1}$, density-1.038 $g \cdot ml^{-1}$, boiling point - 247.1 °C; melting point−21 °C.

Surface activity of the synthesized substances was investigated at the air-water interface by tensiometer "KSV Sigma 702" (Finland) using Du Nouy ring [6]. The method consists in measurement of the maximum force required for detaching the ring from the liquid surface. Orthophophoric acid was used as the product of "Component-Reactant" Joint Stock Company (Moscow, Russian Federation) which is 86% wt. aqueous solution

Water was used as a bidistillate.

Specific electroconductivity (κ) of the aqueous solutions of the obtained surfactants was measured by "Anion-4120" electroconductometer (Russian Federation).

IR-spectra were registered by Vertex 70 (Bruker) spectrometer in the range 4000-400 cm^{-1} using KBr tablets.

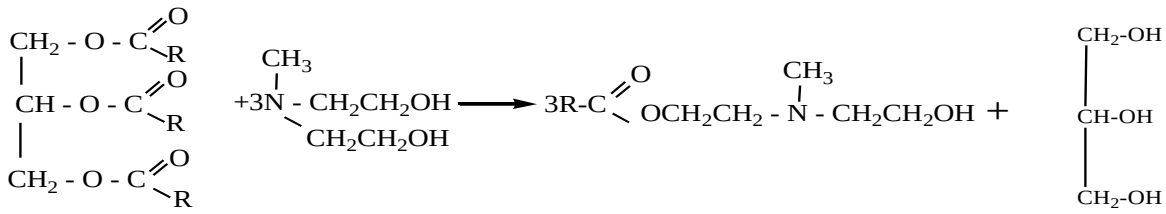
Aminoester was synthesized on the basis of cotton-seed oil triglycerides and MDEA at 140-150ºC during 13-14 hours in an autoclave equipped with a temperature regulator. Subsequently, phosphation of the synthesized aminoester was carried out by reacting

the aminoester with orthophosphoric acid at 50-60ºC during 5-6 hours. Both surfactants are brown viscous liquids.

To study antimicrobial properties of the synthesized surfactants, the method of consequent dilution was used. The experiments were carried out in the test tubes which contained a surfactant at different concentrations and 1ml of the physiological solution. The 1-st test tube is filled with 1ml of 1% wt. solution of the surfactant. Afterwards, the solutions of the surfactant were prepared in the order of concentrations lowering twice. With this aim, the content of the 1-st tube was stirred and 1ml of it was introduced into the 2-nd tube, from the 2-nd into the 3-rd one and so on. From the last tube, 1ml was taken out to have equal volumes of the solutions in all tubes. In this way, four consequently diluted solutions of the surfactant were prepared at dilutions 1:100 (solution 1), 1:200 (solution 2), 1:400 (solution 3)and 1:800 (solution 4) at equal volumes of the physilogical solution. As a test culture, two gram positive bacteria (Staphylococcus aureus and spore forming BacillusAntracoides), three gram-negative bacteria (Pseudomonas aeruginosa, Escherichia coli and capsule forming Klebsiella pneumoniae) and fungus (Candida albicans) laboratory strains were applied. From 1 day culture of each microorganism, suspensions were made in the physiological solution provided that 1ml of the suspension contains 1 bln of microbacterial cells. After dilution procedure, with Pasteur pipette, 1 drop of microbial suspension having 500 mln microbial cells in 1 ml was added to each test cube. After 10,20,40 and 60 min exposition, the samples were taken away from each test tube using a bacteriological loop and inoculated onto the surface of the feeding media (for bacteria-meaty-peptonian (peptone) agar and for fungus- Sabouraud medium). Incubation for fungus was performed in the thermostat at 28^{O}C, for 24-48 h, for bacteria-at 37^{O}C for 24 h. Intensive growth of a microorganism was noted as "+", growth of separate colonies-by the mark "+-" and absence of the growth of a microorganism-by the sign"-".

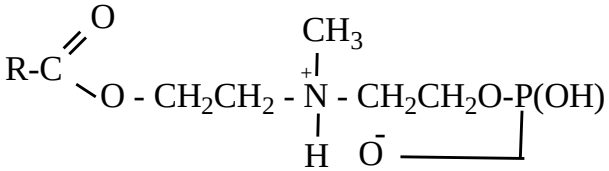
RESULTS AND THEIR DISCUSSION

The scheme of the interaction of cotton-seed oil triglycerides with MDEA is described as follows:



where R is alkyl group glycerol was removed from the mixture by washing with cold water. The final aminoester is soluble in water and kerosene.

In the next step,the MDEA ester obtained from the cotton-seed oil triglycerides was phosphated with orthophosphoric acid. The chemical formula of the synthesized surfactant is illustrated as following:



where R is a saturated or unsaturated hydrocarbon group. The reaction product is a brown substance of low viscosity.

Structure and composition of the obtained products were confirmed by using IR-spectroscopy. The IR-spectra are shown in Fig. 1 and 2.

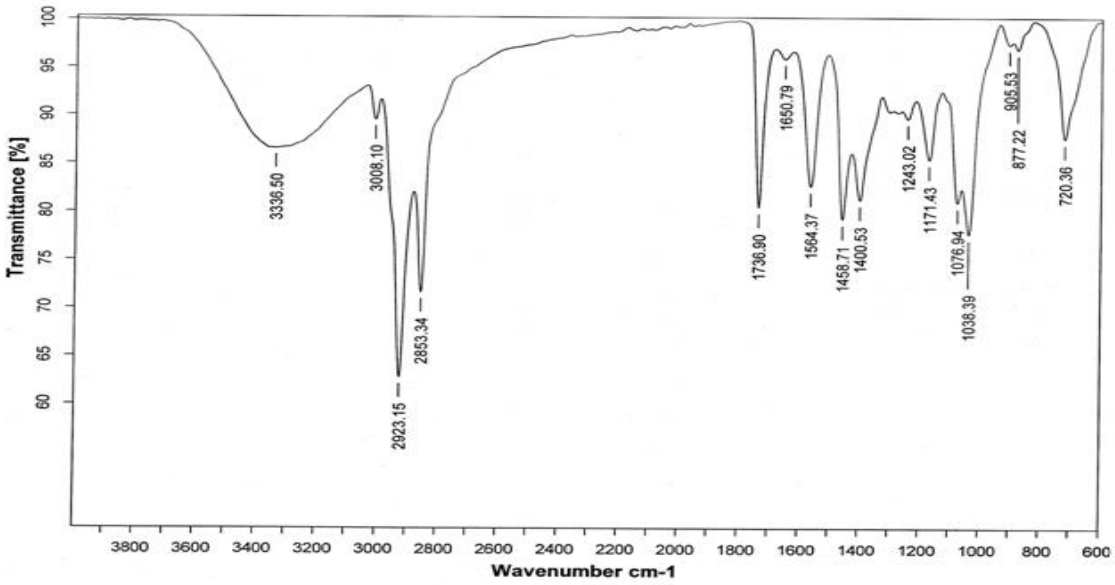


Fig. 1. IR-spectrum of MDEA ester synthesized from cotton-seed oil triglycerides

İn the IR-spectrum of MDEA ester (Fig.1), the following bands (ν_{max}, cm^{-1}) are present: 3336.50 (O-H valence vibration band, 3008.1 (C-H valence vibration band of the double bond), (2923.2 C-H valence vibration band of -CH_2- group) and 2853.3 (valence vibration band of C-H in CH_3 group), 1736.9 (valence vibration band of carbonyl group of ester fragment, 1650,8 (valence vibration band of C=C), 1564.4 (deformation vibration band of OH) and 1400.5 (deformation vibration bands of C-H in CH_2 and CH_3), 1243.0 (valence vibration band of C-N), 1171.4 and 1076.9 (ester group deformational vibrations bands), 1038.4 (valence vibration band of C-O in COH), 720.4 pendulum vibration band of (CH_2).

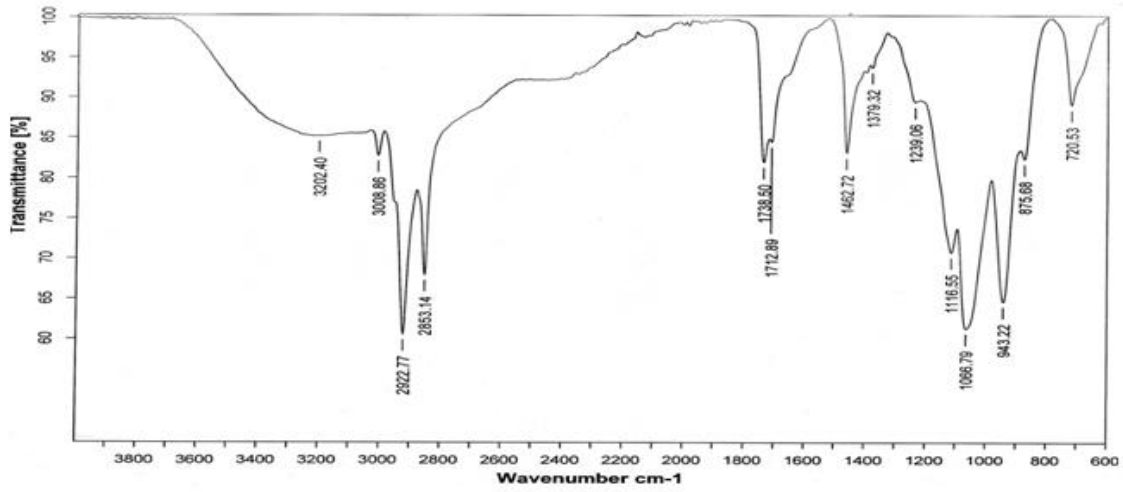


Fig. 2. IR-spectrum of the phosphate derivative of MDEA ester based on cotton-seed oil triglycerides

In the IR-spectrum of the phosphate of MDEA ester, the following changes are observed (ν_{max}, cm^{-1}): the band at 3202.4 (O-H valence vibration band of the phosphate group) appears and the band at 3336.50 of OH valence vibrations disappears; the bands at 2100-2700 of $\overset{+}{N}H$ group appear; the band 1564.4 of OH-deformational vibrations and 1038.4 (valence vibration band of C-O band of C-OH group) disappear; the bands at 1116.6 of valence vibrations of P-O band in P-OH group and at 943.2 of valence vibrations of C-O band in C-O-P group appear.

The data of the given IR-spectra prove proceeding of modification with H_3PO_4.

MDEA ester and its phosphate have a good solubility in isopropanol, isooctane, kerosene, benzene, carbon tetrachloride, a partial solubility in ethanol and water.

Surface tension data of surfactants 1and 2 were recorded at 25 and 26ºC, respectively, γ versus concentration -c plots of the surfactants are given in Fig. 3.

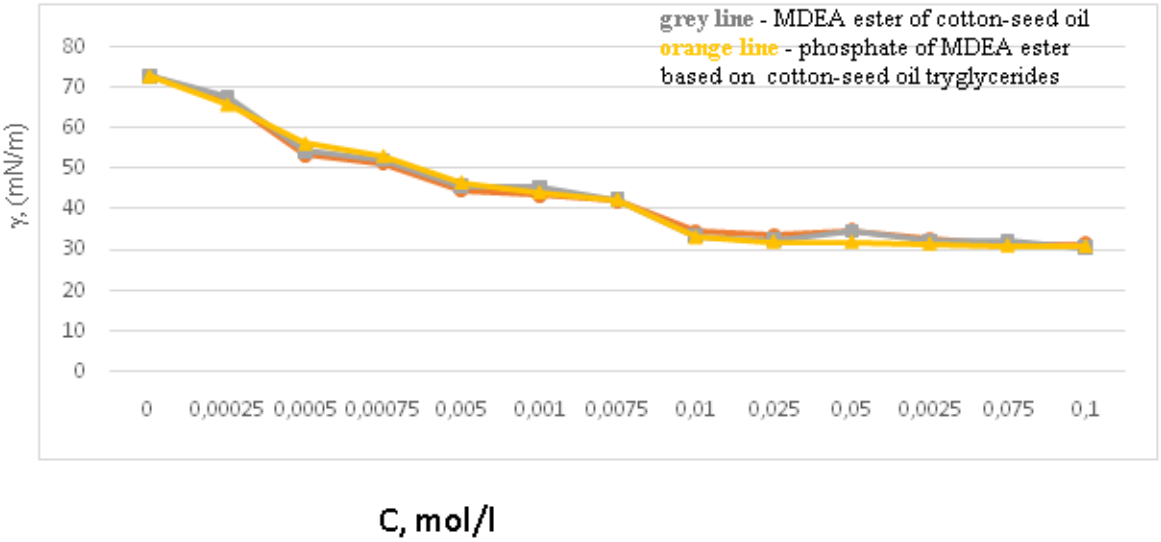


Fig. 3. Surface tension at the water-air interface versus concentration plots of the MDEA ester (grey line; 25 °C) and its phosphation derivative (orange; 26 °C)

Using these isotherms, important parameters of the surface activity may be determined. The values of critical micelle concentrations (CMC) of the surfactants were found. Moreover, γ_{CMC}, surface pressure (π_{CMC}), C_{20} (the concentration for reduction of γ by 20 mN/m), adsorption efficiency ($pC_{20} = -logC_{20}$) values for surfactants were calculated according to [6] and given in Table 1.

Maximum adsorption-Γ_{max}values were determined by the following equation:

$$\Gamma_{max} = -\frac{1}{n * R * T} * \lim_{c \to c_{CMC}} \frac{d\gamma}{dlnc}$$

where R is universal gas constant (R=8.3145 C/mol*K) and T is absolute temperature. The value of n was taken as 2 because 2 ions are formed by dissociation of the surfactants (explained later page).

The minimal value of the area per surfactant molecule after adsorption at the water-air interface (A_{min}) was calculated from the equation

$$A_{min} = \frac{10^{16}}{N_A \times \Gamma_{max}}$$

and given in Table 1.

Specific electrical conductivity versus concentration plot was built for MDEA ester at 27 °C and for the phosphate of MDEA ester - at 27.5 °C. These dependence are given in Fig. 4:

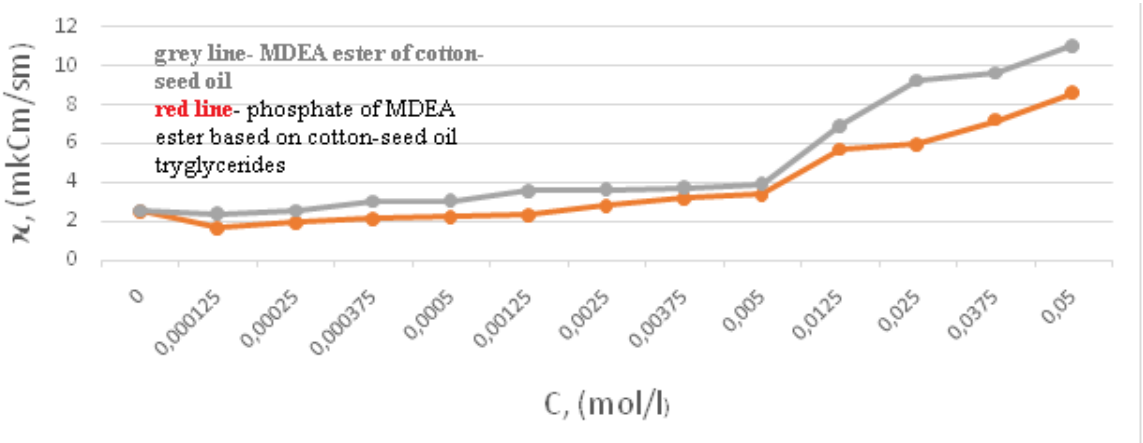


Fig. 4. Specific electrical conductivity versus concentration plots of the synthesized surfactants: grey line- MDEA ester, red line- phosphate of MDEA ester

Despite the fact that the structure of MDEA ester is non-ionic, its aqueous solution has electrical conductivity. This is explained by the fact that tertiary amine fragment reacting with water forms the ionic structure according to this scheme:

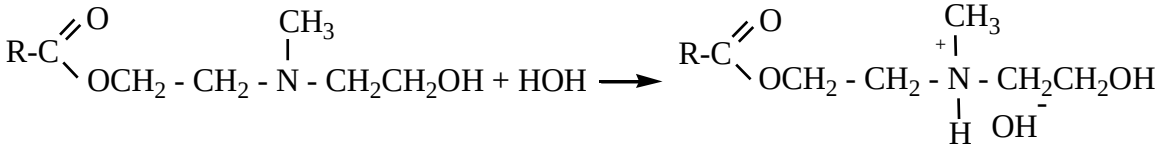


Table 1.

Main colloidal-chemical and thermodynamic parameters of the obtained surfactants

Surfactant	CMC, mM^{-1}	Γ_{CMC}, mN m^{-1}	Π_{CMC}, mNm^{-1}	Γ_{max},x10^{-10} $mol{\cdot}m^{-2}$	A_{min}, nm^2	α	β	ΔG^{o}_{mic}, kJ· mol^{-1}	ΔG^{0}_{ads}, kJ ·mol^{-1}
MDEA ester based on cotton-seed oil	0.034	27.50	45.50	1.40	1.41	0.33	0.67	-25.71	-29.53
Phosphate of MDEA ester	0.01	36.92	33.08	0.55	2.03	0.25	0.75	-91.46	-60.17

Slopes of the straight line before (S_1) and after (S_2) CMC value for each surfactant were determined. Such important thermodynamic indices as Gibbs free energy of micellization (ΔG_{mic}) and Gibbs free energy of adsorption (ΔG_{ad}) values were determined by the following equations:

$$\Delta G_{mic} = (2 - \alpha) \times R \times T \times \ln(CMC)$$

$$\Delta G_{ad} = (2 - \alpha) \times R \times T \times \ln(CMC) - 0.6023 \times \pi_{CMC} \times A_{CMC}$$

where A_{CMC} is surface area per surfactant molecule at the interface in terms of $Å^2$.

Degree of dissociation – α and degree of the counter-ion binding –β were computed by the formulas:

$\alpha = S_2/S_1$ and $\beta = 1-\alpha$

As is evident, the ΔG_{ad} values are more negative than the ΔG_{mic} values which indicates a preference of the adsorption of the surfactant molecules rather than their micelle formation.

The results of studies of antimicrobial properties of MDEA ester and its phosphate derivative are set in Table 2. These results show that MDEA ester is effective against Pseudomonas aeruginosa at each exposure time and each dilution. Against Klebsiella pneumoniae, an effectiveness is exhibited at all times of exposure in the case of 1:100 and 1:200 dilution. At dilution 1:400 and 1:800, the bacterium is suppressed after 40 min of exposure. Against Staphylococcus aureus, a bactericide action is manifested at each degree of dilution after 40 min of exposure.

The phosphate of MDEA ester shows an effect against Esherichia coli and Pseudomonas aeruginosa at all times of exposure at dilution 1:100 and 1:200. In the case of dilution 1:400 and 1:800, a bactericide action is displayed after 40 min. Against Bacillus antracoides, at dilutions 1:100, 1:200 and 1:400, a bactericide effect is observed at all exposure times but at dilution 1:800- after 10 min. In the case of Klebsiella pneumoniae, the following results are found. At dilution 1:100, an effect is manifested at all exposure times, at dilution 1:200- after 20 min, at dilution 1:400-after 40 min and, in the case of dilution 1:800- only after 60 min. The phosphate also exhibits moderate antifungal properties against Candida albicans. An effect is observed at all dilution degrees after 40 min.

Table 2.

Results of study of antimicrobial properties of MDEA ester (1) and its phosphate (2)

Test-culture	Exposure time (min)	Tested substances							
		1				2			
		1	2	3	4	1	2	3	4
Staphylococcus aureus	10	+	+	+	+	+	+	+	+
	20	+	+	+	+	+	+	+	+
	40	-	-	-	-	±	±	+	+
	60	-	-	-	-	±	±	+	+
Esherichia coli	10	-	-	-	-	-	-	+	+
	20	-	-	-	-	-	-	+	+
	40	-	-	-	-	-	-	-	-

	60	-	-	-	-	-	-	-	-
Pseudomonas aeruginoza	10	-	-	-	-	-	-	+	+
	20	-	-	-	-	-	-	+	+
	40	-	-	-	-	-	-	-	-
	60	-	-	-	-	-	-	-	-
Candida albicans	10	+	+	+	+	+	+	+	+
	20	+	+	+	+	+	+	+	+
	40	+	+	+	+	-	-	-	-
	60	+	+	+	+	-	-	-	-
Biccillus antracoides	10	+	+	+	+	-	-	-	+
	10	+	+	+	+	-	-	-	+
	20	+	+	+	+	-	-	-	-
	40	+	+	+	+	-	-	-	-
	60	+	+	+	+	-	-	-	-
Klebsiella pn.	10	-	-	+	+	-	+	+	+
	20	-	-	+	+	-	-	+	+
	40	-	-	-	-	-	-	-	+
	60	-	-	-	-	-	-	-	-

Note: 1,2,3,4- 1: 100, 1: 200, 1: 400, 1: 800; "+" Indicates “growth”; "-" indicates “suppression”, “±” indicates existence of colonies

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