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SYNTHESIS AND SELFASSAMBLING OF AMPHIPHILIC OLIGOESTERS BASED ON PYROMELLITIC ACID

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Abstract. The method for synthesis of a new class of amphiphilic oligoesters of pyromellitic acid is developed. As hydrophilic fragments polyethylene glycols or polyethylene glycol mono methyl ethers were used, as lipophilic ones – primary fatty alcohols or cholesterol. The structures of the synthesized oligoesters were confirmed by IR- and PMR-spectroscopy. The oligoesters could solubilize water-insoluble substances, for example such effective antitumor lipophilic drug as curcumin. The high solubilization capacity of the oligoesters assemblies and their biodegradability, as well as other properties (size distribution, ζ-potential) make the oligoesters considered as promising materials for the design of drug delivery systems.

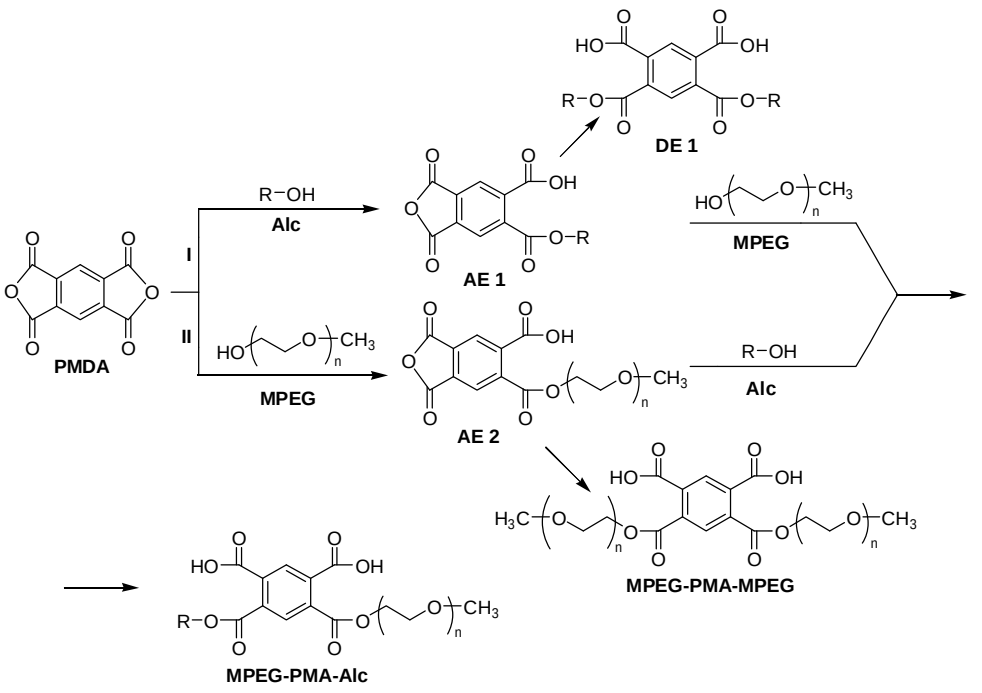
Keywords: pyromellitic acid, polyethylene glycol, oligoester, cholesterol, solubilisation, curcumin.

1. Introduction

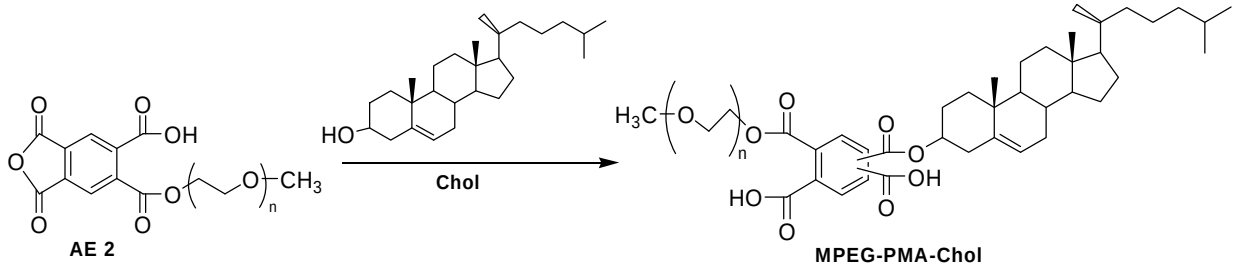
A topical problem of modern pharmacology is development of nanoscale platforms for delivery of hydrophobic drugs to teratoid cells. To solve this problem amphiphilic polymers and oligomers that can form self-assembled associates such as micellar structure [1-3], micro- and nanocapsules [4], and liposomes [5] are used. They are able to solubilize certain number of molecules of drug substance and protect them against rapid filtration in the kidneys, destruction by proteases, esterases and other enzymes, thereby increasing the circulation time in the blood vessel [6].

New approach to creation of amphiphilic surfactants is connected with using a pyromellitic acid (PMA) as a reagent for synthesis. PMA is quadribasic aromatic acid. Due to the presence of reactive carboxyl groups it can form a surfactants molecule with predetermined hydrophilic-lipophilic balance through interaction with relevant lipophilic and hydrophilic alcohols. It was expected that consecutive interaction of pyromellitic dianhydride (PMDA) with hydrophilic polyethylene glycol (PEG) or polyethylene glycol monomethyl ether (MPEG) and lipophilic primary aliphatic alcohol (Alc) or cholesterol (Chol) in accordance with Schemes 1-4 would results in the following new amphiphilic oligoesters of pyromellitic acid (OEPA).

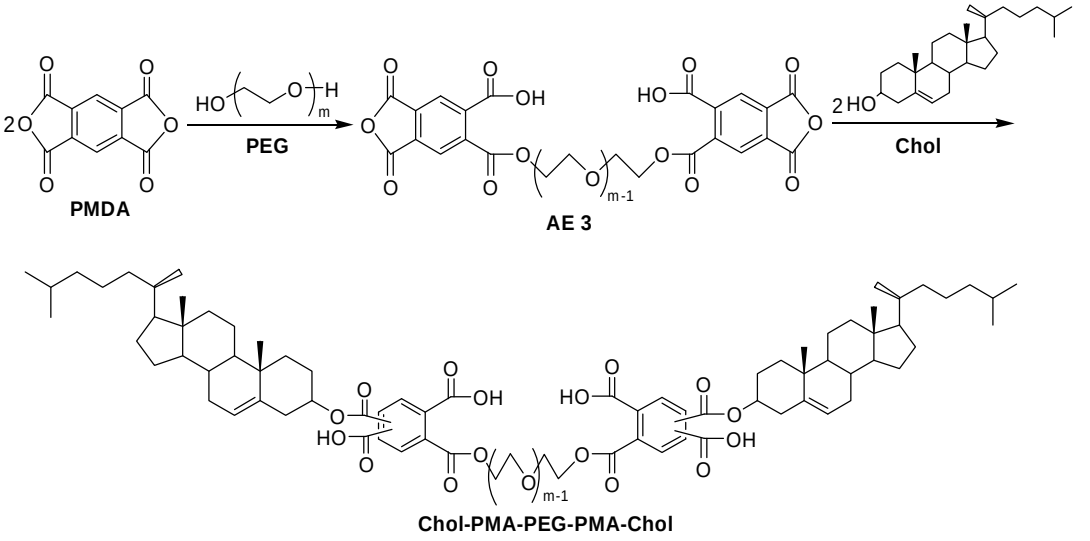
Choosing cholesterol as lipophilic alcohol was caused by the fact that cholesterol is a key component of eukaryotic cell membranes and is characterized by high thermodynamic affinity of their surface. The molecules of cholesterol affect stability and permeability of the double lipid layer of cell membranes [7, 8], regulate the activity of membrane proteins, including multiple receptors and ion channels, *etc*. [9-11]. Water-soluble derivatives of cholesterol can self-assemble to form aggregates, micelles and liquid crystalline phases [12-16]. It is generally anticipated that the hydrophobic nature of cholesterol moieties facilitates a broad variety of polymeric self-assemblies in an aqueous medium [17]. To synthesize water-soluble derivative of cholesterol and generate their self-assembly in an aqueous medium, one can combine fragments of cholesterol with hydrophilic ones – for example, PEG. The PEGs are highly biocompatible and



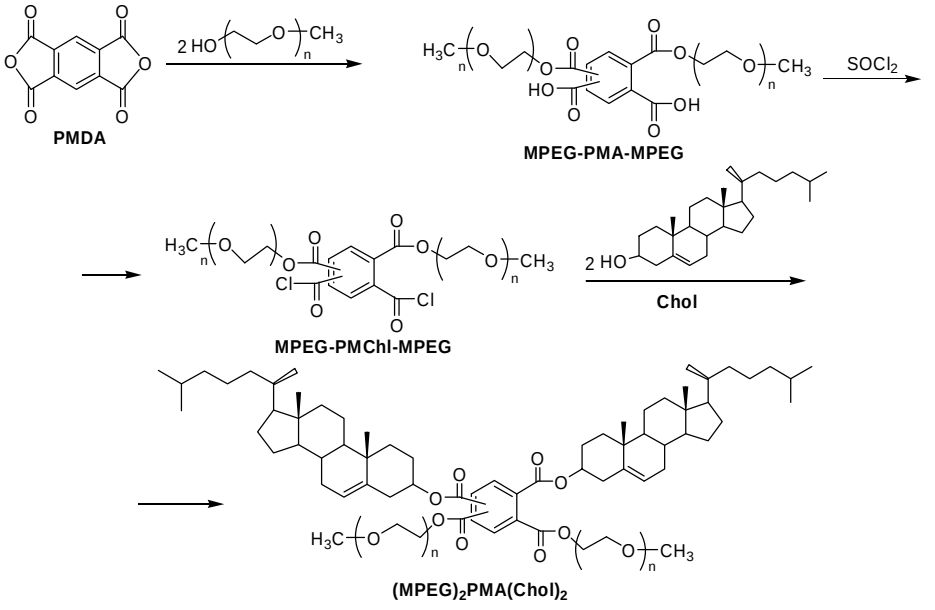
Scheme 1



Scheme 2



Scheme 3



Scheme 4

where R = C_8H_{17}, $C_{16}H_{33}$; n = 7, 12, 16; m = 13

provides stealth behavior by decreasing the interaction with blood, thus making it the only polymer currently approved as a therapeutic agent at the point of drug delivery [18]. Also it was expected that the structural features of the obtained oligomers provide them with the ability to solubilize controlled amounts of poorly water-soluble substances in an aqueous medium. Developed new synthetic routes (Schemes 1-4) allow varying the chemical structure of oligoesters and constructing nanocariers with desired properties. Additionally, the cholesterol moieties are expected to affect the interaction between carriers and the biomembranes. These carriers will also affect pharmacokinetics of drugs substances due to protection against hydrolysis and attack of immune system. At the same time, oligoesters are able to undergo hydrolysis and remove from an organism.

The purpose of this work was to develop a new synthetic route for attachment of hydrophilic PEG and lipophilic alcohol/cholesterol fragments in a controllable manner to pyromellitic acid in order to obtain amphiphilic oligoesters. Further goal was to demonstrate the self-assembly and ability of the new oligomers to solubilize poorly watersoluble molecules in an aqueous medium.

2. Experimental

2.1. Reagents and Materials

PMDA (97 %, Sigma Aldrich) was purified by sublimation in a vacuum at a residual pressure of 5–10 mm Hg and kept in a desiccator over calcium chloride. Melt. point 561–562 K (lit. 556–559 K [19]), acid number 1036 mg KOH/g (calc. 1029 mg KOH/g). Mono methyl ethers of polyethylene glycols (Aldrich) M_w = 350 (MPEG 350), M_w = 550 (MPEG 550), M_w = 750 (MPEG750) and polyethylene glycol (Aldrich), M_w = 600 (PEG 600) were dried under vacuum in a stream of argon at 373–393 K. Pyridine was dried over anhydrous sodium hydroxide, filtered and distilled at atmospheric pressure under argon; boiling point 387 K (lit. 388 K [20]), n_D^{20} = 1.5088 (lit. 1.509 [20]). Dichloromethane, 1,2-dichloroethane (DCE), hexane, dimethylformamide (DMF), dimethylsulfoxide (DMSO), 1-octanol (Sigma Aldrich) were purified in accordance with techniques described in [20]. Cholesterol (Sigma, Cat. No. S75209), cetyl alcohol (Aldrich), curcumin (Overseal Natural Ingredients Ltd), 4-methyl-2-pentanone (Sigma Aldrich, ACS reagent, ≥ 98.5 %), triethylamine (TEA) (Sigma Aldrich, ≥ 99 %) were used without further purification. Thionyl chloride (TChl) (Sigma) was distilled at atmospheric pressure in a stream of argon. *Tert*-butyl hydroperoxide (TBHP) was prepared using alkylation of hydrogen peroxide with *tert*-butyl alcohol [21] and purified by fractional vacuum distillation at 19.5 kPa. n_D^{20} = 1.4011 (lit. n_D^{20} = 1.4013); active oxygen content of 17.6 % (calc. 17.7 %).

2.2. Kinetic Studies

The calculated amount of PMDA was loaded in round-bottom reactor (50 ml capacity) and dissolved in

certain amount of DMF. The flax was placed into constant-temperature bath for 10 min, and a sample of TBHP, heated up to the same temperature was added under vigorous agitation. To monitor the reaction, a number of samples (1 ml) were collected as the reaction progressed, into 50 ml water (stopper), and titrated with 0.1 N KOH solution or TBHP content was determined as described in [22].

For NMR spectroscopy kinetics studies 0.177 g (0. 812 mmol) PMDA was dissolved in 2 g of anhydrous deuterated DMSO at 373 K, and then cooled to 303 K. The ampoule with this solution was placed into a thermostat at 303 K for 10 min and 0.163 g (1.81 mmol) TBHP were injected. After regular intervals ^{1}H NMR spectra were recorded for plotting the integrated signal intensity *via* time.

2.3. Synthesis MPEG-PMA-Alc

Route I: 10.9 g (0.05 mol) PMDA were dissolved in 70 g of dry DMF, 0.2 g (≈ 2 mmol) TEA were added and under vigorous stirring at 353 K a solution of 0.05 mol of 1-oktanol/cetyl alcohol in 20 ml of DMF was added drop wise during 5.6 h. The mixture was stirred for 24 h at 353 K. Afterwards, 0.05 mol MPEG were added and stirred for a further 24 h at 353 K. Reaction conversion (97–99 % at both stages) was controlled by the content of carboxyl groups, determined by pH-metric titration of 0.6 ml of reaction mixture (diluted in excess DMF) by 0.1 N aqueous sodium hydroxide. After the synthesis, DMF was distilled from the reaction mixture using a rotary evaporator. Alc-PMDA-MPEG esters were purified by triple precipitation from the acetone solution into hexane, and washed using 4-methyl-2-pentanone to remove any side products, as well as MPEG and PMDA residues (final product yield 32–38 %). Purified OEPA were dried at 328 K. Identified by thin-layer chromatography on the plates "Silufol", eluent – benzene.

Route II: 10.9 g (0.05 mol) PMDA were dissolved in 70 g of dry DMF, 0.2 g (≈ 2 mmol) TEA were added and under vigorous stirring at 353 K a solution of 0.05 mol of MPEG in 20 ml of DMF was added drop wise during 5.6 h. The mixture was stirred for 24 h at 353 K. Afterwards, 0.05 mol of 1-oktanol/cetyl alcohol were added and stirred for a further 24 h at 353 K. Control of the conversion, isolation, purification and identification of OEPA was performed as described above. Yield 29–36 %.

2.4. Synthesis MPEG-PMA-Chol

10.9 g (0.05 mol) PMDA were dissolved in 70 g of dry DMF, 0.2 g (≈ 2 mmol) TEA were added and under vigorous stirring at 353 K a solution of 0.05 mol of MPEG in 20 ml of DMF was added drop wise during 5.6 h. The mixture was stirred for 24 h at 353 K. Afterwards, 19.3 g (0.05 mol) of dry cholesterol were added and stirred at 353 K until the degree of conversion, which was determined by pH-metric method, reached 97–99 % (about 25 h). After distilling off the solvent under vacuum PEG-PMA-Chol was dissolved in acetone and solution was filtered to remove residual cholesterol and cholesteryl pyromellitate esters (insoluble in acetone). The product was precipitated by hexane and triply precipitated from acetone solution into hexane. The precipitate was dried, dissolved in 5 % aqueous sodium carbonate solution, precipitated by adding 5 % HCl solution and excess of saturated NaCl solution and dried under vacuum at 353 K. Yield 30–40 %.

2.5. Synthesis Chol-PMA-PEG-PMA-Chol

13.1 g (0.06 mol) PMDA were dissolved in 85 g of DMF, 0.2 g (≈ 2 mmol) TEA were added and slowly under vigorous stirring at 353 K a solution of 17.1 g (0.028 mol) PEG-600 in 40 ml of DMF was added drop wise during 5.6 h. The mixture was stirred for 24 h at 353 K, then 27.0 g (0.07 mol) of dry cholesterol were added and mixture was stirred at this temperature to achieve the degree of conversion of 97–99 % (about 25 h). Isolation, purification and identification of OEPA were performed as for the preparation of MPEG-PMA-Chol. Yield 27.3 g (54 %).

2.6. Synthesis $(MPEG)_2PMA(Chol)_2$

Diesters of Pyromellitic acid and MPEG-550 (MPEG-PMA-MPEG). 9.1 g of MPEG-550 (0.0165 mol), 1.8 g (0.0082 mol) of PMDA, 0,1 ml of pyridine were loaded into reactor and stirred for 11 h at 373 K. The product was dissolved in 20 ml dichloromethane and precipitated with 40 ml of hexane. After triple reprecipitation MPEG-PMA-MPEG was dried in a vacuum of 2.6–6.5 kPa at 353 K for over 3 h. Yield was 10 g (92 %) MPEG-PMA-MPEG as a yellowish transparent resin. Acid number 87.9 mg KOH/g (calc. 85.1 mg KOH/g).

Chloro anhydride of Pyromellitic acid and MPEG-550 ester (MPEG-PMChl-MPEG). 7.5 g (0.0057 mol) of MPEG-PMA-MPEG and 10 ml of $SOCl_2$In were placed into reactor, equipped with a sealed stirrer, thermometer and backflow condenser, connected with a scrubber to absorb the acid vapor charged. The reaction mass was boiled for 10 h at an oil bath, then an excess of $SOCl_2$ was distilled off, residue was dried under vacuum and 7.8 g (quantitative yield) of a product as orange transparent liquid was obtained. Acid number 170 mg KOH/g (calc. 165.6 mg KOH/g).

$(MPEG)_2PMA(Chol)_2$. The reactor was loaded with 30 ml of DCE, 2.3 g (0.006 mol) of cholesterol and 0.47 g (0.006 mol) of pyridine. The reaction mass was cooled

down to 278 K and a solution of 4 g (0.003 mol) MPEG-PMChl-MPEG in 20 ml of DCE was added drop vise at 278–281 K, stirred for 1 h at 278 K and for another 3 h at 293 K. The reaction mass was washed with a small amount of 2 % HCl solution, 5 % solution of sodium carbonate, and then with water. The lower organic layer was separated and the solvent was distilled off in vacuum at 323 K. The residue was dissolved in methanol and insoluble cholesterol was filtered. The target product was twice reprecipitated with hexane from acetone solution. 2.4 g (38 %) of product (a waxy substance) was yielded after drying.

2.7. Spectroscopy

The FTIR spectra of synthesized oligoesters were recorded in a thin layer deposited from the benzene solution on a potassium bromide tablet, using a Thermo Scientific Nicolet Fourier Transform Infrared Spectrometer, in the range of 400-4000 cm^{-1} with compensation of atmospheric CO_2 and H_2O. ESI high-resolution mass spectra were obtained using "Bruker Daltonics BioTOF" mass spectrometer. ^1H NMR spectra were recorded in acetone-d_6 and chloroform-*d* solutions using a 500 MHz "Varian Inova" spectrometer.

2.8. Surface Tension to Determine the Critical Micelle Concentration (CMC) of OEPAs

The surface tension measurements were performed by drop shape analysis on pendant drops with a Contact Angle/Surface Tension Analyzer, First Ten Angstroms (FTA 125) to determine the CMC of the DEPAs. Measurements were carried out at 398 K for the oligomer solutions of various concentrations (10^{-7}–1 wt %). Aqueous DEPA solutions were prepared by dissolving the DEPA in bidistilled water and further adjusting the pH using a 15 % aqueous sodium carbonate solution. All glassware was washed in 1 N NaOH bath and thoroughly rinsed with Millipore water before use.

2.9. Fluorescence Spectroscopy to Determine CMC of DEPAs

Alternatively, the CMC of OEPAs was measured using the solubilization of a fluorescent probe, pyrene, using the method [23, 24]. The spectra were taken using a Fluoromax-3 Fluorescence Spectrometer (Jobin Yvon Horiba) with 90° geometry, and a slit opening of 0.5 nm. For fluorescence excitation spectra, λ_{em} = 390 nm was chosen. Spectra were accumulated with an integration time of 0.5 nm/s. Critical micelle concentration values were determined after fitting the semi-logarithmic plots of intensity ratio $I_{336.5}/I_{333,5}$ *vs.* log concentration to the sigmoidal curve.

2.10. Curcumin Solubilization in OEPA Micelles

To determine curcumin solubilization, a sample of curcumin powder was mixed with different oligomer concentration solutions in a phosphate buffer (pH 6.58), and stirred at room temperature for 48 h. Excessive curcumin was filtered. The UV-vis spectra of curcumin-solubilized oligomer solutions were recorded using Varian Cary 5000 at 293 K. The concentration of solubilized curcumin was determined from the absorption spectra at characteristic 470 nm wavelength, using a calibration curve. The calibration curves were built after recording the UV-vis spectra of a set of curcumin solutions of known concentrations in the 1-octanol.

2.11. Solubilization of Non-Polar Solvents in DEPA Micelles

To study the solubilization of hexane/benzene in DEPA micelles, 0.01 ml of hexane/benzene was gradually added, every 2 days, to 20 ml of aqueous oligomer solution. The optical density, *D*, of the DEPA aqueous solutions were measured using the Photoelectric Colorimeter K 77 (λ = 535 nm) every two days, before adding a new portion of hexane/benzene. The procedure was repeated 10-15 times until the solution became turbid. The experimental values of *D* were plotted *vs.* the volume of hexane/benzene added and the solubilization amount was determined as a slope change.

2.12. The Size Distribution and the Zeta Potential of Oligomeric Assemblies

The size distribution and the zeta potential of oligomeric assemblies were measured using a Malvern Zetasizer Nano-ZS90 at 298 K and pH 6.5 ± 0.05. The final numbers represent an average of a minimum of five (size) or ten (zeta potential) individual measurements.

3. Results and Discussion

3.1. Kinetics of Acylation Reaction of any Alcohol with PMDA

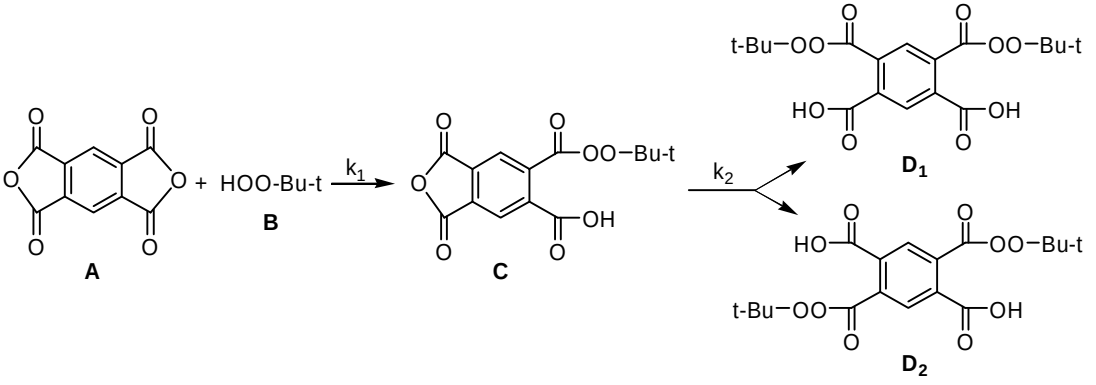
In accordance with Schemes 1–3 OEPA synthesis consists of two consecutive stages: 1 – obtaining of monoesters (AE 1-3), which contain residual reactive anhydride group and 2 – their interaction with alcohol or cholesterol to form targeted OEPA. The success of the synthesis depends on the ability to preserve in the molecules of intermediate monoesters (AE 1-3) reactive

anhydride group. Therefore it is important to investigate the kinetics of acylation reaction of any alcohol with PMDA to determine the factors which promote the maximum yield of intermediate anhydride monoester. As model system the reaction of PMDA with TBHP (Scheme 5) was used, because of possibility to control the content of initial hydroperoxide (**B**) and formed peroxyesters (**C**, **D_1**, **D_2**) through the definition of hydroperoxide and peroxyester active oxygen [22]. In addition, the overall rate for the process can be controlled by acid number measurement, and the content of each component could be controlled using the PMR spectroscopy method.

It is known that the reactions of monobasic carboxylic acids anhydrides with alcohols are of a general second order (first for anhydride and first for alcohol [25]). However, the kinetic curves depicted in Fig. 1 demonstrate a significant retarding of reaction after reaching 27–34 % conversion. These curves could not be described by kinetic equations of neither first nor second order and indicate that this reaction proceeds by more complicated mechanism.

The reaction orders for individual reagents were determined using the Method of Initial Velocities [26]. The calculated values are 0.95 for TBHP and 2.12 for PMDA. Thus, this process is described by the following kinetic equation: $V = k[\text{TBHP}][\text{SPMDA}]^2$. General third order is confirmed by a linear character of kinetic curves W_A/C_A^2 *via* C_A, where W_A – the rate of anhydride groups exhausting, C_A – total concentration of anhydride groups in the reaction mass (Fig. 2), and this relationship persists up to conversion of about 40 %.

Probably PMDA additionally acts as a catalyst of the process. The catalytic effect of PMDA can be explained by a general acid catalysis due to the carboxyl groups formed during the reaction. A similar phenomenon has been described by Antonovskiy [27] for acylation of TBHP with acetic anhydride in the presence of acetic acid. Consequently, catalytic effect of PMDA could be repressed by realizing base catalysis mechanism. It can be seen from Fig. 3 that in the presence of pyridine (base catalysis) initial velocity of reaction does not depend on the PMDA concentration, indicating a zero order for PMDA.



Scheme 5

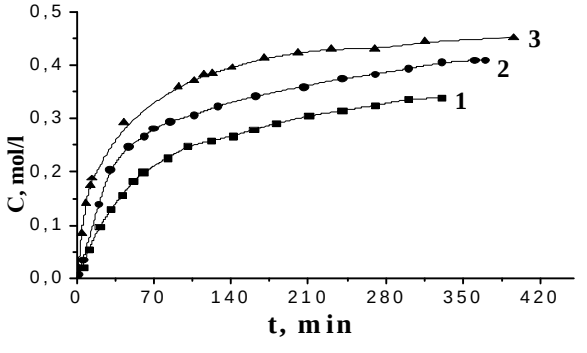


Fig. 1. Kinetic curves representing overall concentration of peroxyester groups in the reaction of PMDA with TBHP in DMF at temperature (K): 303 (1); 313 (2) and 323 (3). C_0(PMDA) = 0.33 mol/l; C_0(TBHP) = 0.73 mol/l

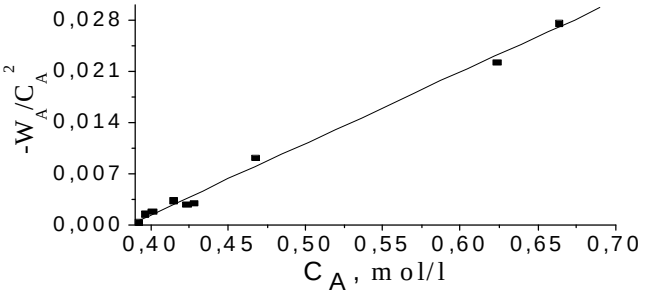


Fig. 2. The normalized rate of reaction TBHP with PMDA *vs*. anhydride groups concentration at 303 K in DMF and C_0(PMDA) = 0.33 mol/l, C_0(TBHP) = 0.74 mol/l

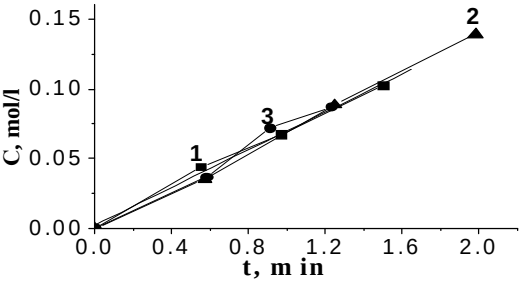


Fig. 3. Kinetics of accumulation of peroxyester groups in the PMDA reaction with TBHP in DMF media at 293 K and C_0(Py) = 0.22 mol/l, C_0(TBHP) = 0.22 mol/l for different C_0(PMDA): 1 – 0.34 mol/l; 2 – 0.30 mol/l; 3 – 0.12 mol/l

To write differential equations of kinetic model we introduce the following notation:

$[A]_0$, $[A]$ – initial and current concentration of PMDA, respectively, mol/l;

$[B]_0$, $[B]$ – initial and current concentration of TBHP, respectively, mol/l;

$[C]$ – current concentration of anhydride monoperoxyester (C), mol/l;

$[D]$ – total current concentration of di*tert*-butylperoxypyromellitates (D_1, D_2), mol/l;

k_1, k_2 – rate constants of the first and second stages.

So we can write the following differential equations for the components of the reaction taking into account experimentally obtained orders for them:

$$\frac{d[A]}{dt} = -k_1[A]^2[B];\ \frac{d[B]}{dt} = -k_1[A]^2[B] - k_2[A][C][B];$$

$$\frac{d[C]}{dt} = k_1[A]^2[B] - k_2[A][C][B];\ \frac{d[D]}{dt} = k_2[A][C][B]$$

After a series of algebraic manipulations, we obtain the final equation:

$$t = -\frac{1}{k_1}\cdot\frac{V-1}{V+1}\cdot\left(\frac{1}{([A]_0+(V-1)\cdot x)^{\left(\frac{1}{V}+1\right)}\cdot[A]_0^{\left(1-\frac{1}{V}\right)}} - \frac{1}{[A]_0^{\,2}}\right)$$

where τ – time, min; V – ratio of constants ($V = k_2/k_1$); x – current concentration of peroxyester groups, mol/l.

This equation allows on the basis of experimental data (Fig. 1) to calculate the values of constants k_1 and k_2 by optimizing them using nonlinear least squares method within the software package "Origin". Optimized constant values for three temperatures are shown in Table 1.

Table 1

The kinetic parameters of PMDA interaction with TBHP

t, K	V	k_1	k_2
303	0.45	0.0025	0.0011
313	0.47	0.0041	0.002
323	0.49	0.0063	0.0031

Visual assessment of the results of the optimization of PMDA interaction with TBHP is possible by comparing the experimentally obtained curves (*curves* 1, Fig. 4) and those constructed based on calculated constants (*curves* 2, Fig. 4).

To test the mathematical model of the process, curves which were constructed based on model and experimental curves obtained from results of PMR spectroscopy (Fig. 5) were compared. Components of the reaction mass (A, C and D) form in PMR spectra signals of aromatic proton in the weak field (Table 2). These signals do not overlap and therefore the ratio between their integrated intensities allows calculating the content of each component in the reaction mixture (Fig. 5).

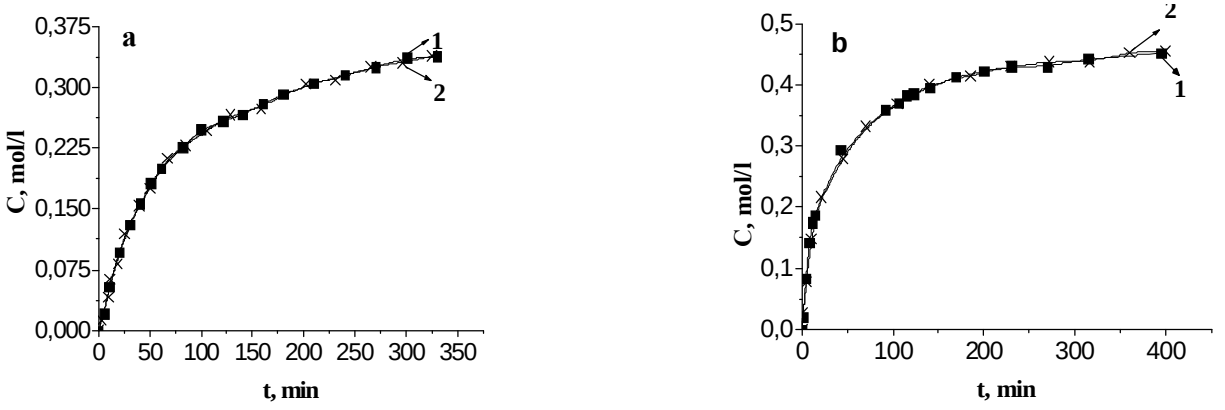
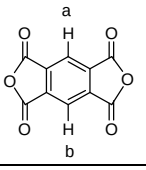
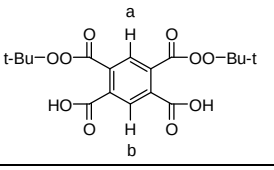
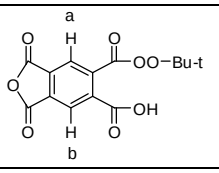
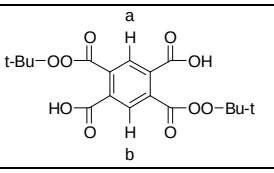


Fig. 4. Kinetic curves accumulation of peroxyesters during PMDA interaction with TBHP at 303 K (a) and 323 K(b): experimental kinetic curve (1) and curve constructed based on calculated constants (2)

Table 2

Characteristics of aromatic protons signals in PMR spectra PMDA and products of their interaction

Product	Proton	Shift, ppm	Character of the signal	Product	Proton	Shift, ppm	Character of the signal
A		*a* = *b* = 8.72	Singlet	D_1		*a* = 8.13 *b* = 7.87	Two singlets
C		*a* = 8.38 *b* = 8.36	Two singlets	D_2		*a* = *b* = 8.04	Singlet

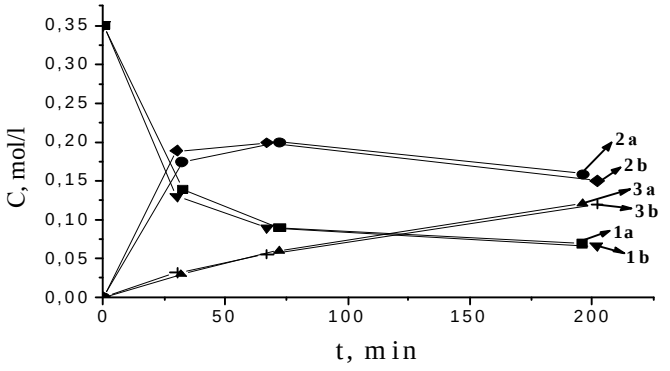
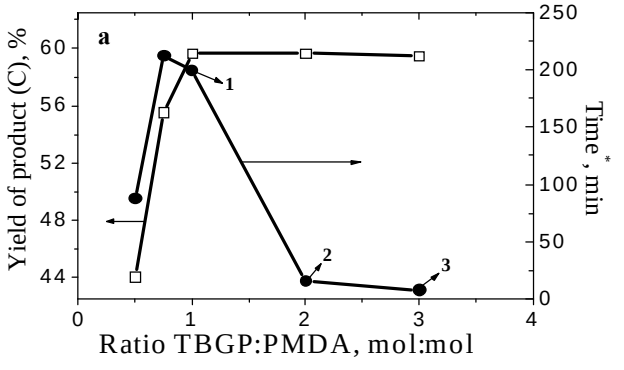


Fig. 5. The kinetic curves of the proce ss of PMDA interaction with TBHP, calculated based on mathematical model (a) and produced according to PMR spectroscopy (b) at 303 K and C_0(PMDA) = 0.35 mol/l, C_0(TBHP) = 0.75 mol/l: reduction of concentration (A) (1); accumulation (C) (2) and accumulation (D_1+D_2) (3)



* – time for reaching maximum yield of C at different ratios TBHP and PMDA

Fig. 6. Effect of the ratio TBHP: PMDA to yield C and duration to reach a maximum yield of C at 303 K (a) and 283 K (b)

Satisfactory conjunction calculated and experimental kinetic curves suggest that the mathematical model sufficiently describes the process. Based on the model the conditions of synthesis were optimized to reach the maximum concentration of product C in the reaction mass. Software package "Maple V R7" was used. The results of this optimization for two temperatures are shown in Fig. 6.

As shown in Fig. 6, the most significant impact on the yield of C is caused by two factors: the ratio of the reagents (TBHP:PMDA) and reaction temperature. It can be concluded that the maximum yield C is reached at equimolar ratio TBHP and PMDA. Temperature decrising also results in higher yield of C, but increases the time to reach maximum yield. Thus, the optimal conditions for obtaining C are as follows: equimolar ratio TBHP and

PMDA and reaction time of 200 min at the temperature of 303 K or 550 min at 283 K.

3.2. Synthesis of Oligomers

3.2.1. Oligomer MPEG-PMA-Alc

It is known that alcohol nucleophilicity is much lower in comparison with TBHP [27] so the PMDA reaction with Alc at 303 K does not occur. To increase the velocity of the process it is advisable to raise the temperature and introduce an effective catalyst, namely TEA. Thus at PMDA interaction with octyl, cetyl alcohol or MPEG at 350 K in the presence of 0.2 % TEA after 24 h almost complete conversion of the initial reagents was achieved.

From Scheme 1 it can be seen that two routes could be used for MPEG-PMA-Calc synthesis: the first (I) consists in PMDA interaction with appropriate alcohol to form anhydrite monoester (AE 1) and its further reaction with MPEG to form MPEG-PMA-Alc; the second route (II) involves acylation of MPEG by PMDA and further interaction of formed monoester (AE 2) with the corresponding Alc. In respect to achieving the maximum yield of target product route II looks more attractive. At the first stage of the second route PMDA interacts with less active MPEG, which possesses lower nucleophilicity than Alc. Therefore, the rate of side reaction (AE 2) with MPEG, which results in diester (DE 2) is less than the rate of interaction (AE 1) with more nucleophilic alcohol to form (DE 1). On the second stage of route II monoester (AE 2) having less reactive anhydride group, than the original PMDA, interacts with more active nucleophile – primary Alc. So the route II is more reasonable to synthesize MPEG-PMA-Alc.

3.2.2. Oligomers MPEG-PMA-Chol and Chol-PMA-PEG-PMA-Chol

Oligomers of this type were received under similar conditions, but the duration of the second stage was greater because of lower reactivity of secondary hydroxyl groups in Chol, in comparison with primary alcohol. Preparation MPEG-PMA-Chol was carried out at equimolar ratio MPEG:PMDA:Chol and Chol-PMA-PEG-PMA-Chol is synthesized if ratio of PEG:PMDA:Chol was 1: 2.1: 2.5 mol, respectively.

3.2.3. Oligomer $(MPEG)_2PMA(Chol)_2$

The process of $(MPEG)_2PMA(Chol)_2$ synthesis consists of three synthetic stages (Fig. 4). At the first stage PMDA reacts with MPEG to form diester (MPEG-PMA-MPEG), which consists of two isomers with different position of carboxyl groups and esteric fragments in benzene ring. At the second stage (DMPP) transforms into acid chloride (MPEG-PMChl-MPEG). The latter possesses active chloranhydride groups, which interact with OH-group in Chol molecule resulting in $(MPEG)_2PMA(Chol)_2$. Unlike previous synthetic schemes, at the first stage of the process both anhydride cycles of PMDA participate in reaction. This makes it possible to carry out the first stage without solvent at higher temperature and stoichiometric ratio of reagents. Products of the second stage acid chloride yield quantitatively and do not require purification since the two by-products of the reaction (HCl and SO_2) are gaseous. The third stage could be carried out at low temperature due to the high reactivity of acid chloride, furthermore low temperature prevents possible hydrolysis of acid chloride by moist air.

The chemical structure of the OEPAs was confirmed using FT-IR and ^{1}H NMR spectroscopy. On FTIR spectra of OEPAs some characteristic bands were marked: 3400–3500 cm^{-1} – OH in COOH groups connected with the aromatic ring; 2931–2927 cm^{-1} – CH_3; 2869–2854, 1467–1456, 760–750 cm^{-1} – CH_2 in alkyl moieties; 1729–1727 cm^{-1} – C=O in the ester fragments; 1616–1612 cm^{-1} – characteristic absorption band of aromatic cycles; 1253–1249 cm^{-1} – C–O (ester); 1106–1105 cm^{-1} – C–O–C link in the PEG fragment; 953–951 cm^{-1} – C–H in the aromatic cycles.

Fig. 7 shows the ^{1}H NMR spectrum of MPEG550-PMA-Alc (Alc = octyl). The ratio of intensities of methyl protons signal in the fragment MPEG (F – 3.54) and octyl (K – 3.48), which is equal to 1.02, confirms the structure of the oligoester.

In the ^{1}H NMR spectrum (Fig. 8) group of signals within 0.65–2.5 ppm is assigned to the protons of cholesteryl fragment. Multiplet at 4.86 ppm depicts cholesteryl fragment proton in the α position to the hydroxylic group (C). The signal at 5.4 ppm corresponds to the proton (B) near double bond in cholesteryl; signals at 0.65, 0,83-0,84, 0,87-0,88 and 1.04 ppm correspond to methylene protons (N), (M), (L), (K), (J), respectively [28]. The ratio of total intensities of signals protons at MPEG-550 (50.92 for 50 protons), cholesteryl (51.31 for 45 protons) moieties and benzene cycle (1.96 for 2 protons) gives the ratio of these moieties (50.92:50)/(51.31:45)/(1.96:2)=1.01/1.14/0.98. Moreover, the values of integral for one proton (C) is 1.07, two protons (D) is 1.94 and two protons (A) is 1.96, further confirming the structure of the product.

The similar signals are shown in the ^{1}H NMR spectrum of $(MPEG)_2PMA(Chol)_2$ (Fig. 9).

The molecular weight of the obtained products was determined by mass spectroscopy method. Mass spectrum of oligomer MPEG550-PMA-Chol is shown in Fig. 8.

Thus, IR and NMR spectra confirmed the structure of synthesized OEPAs.

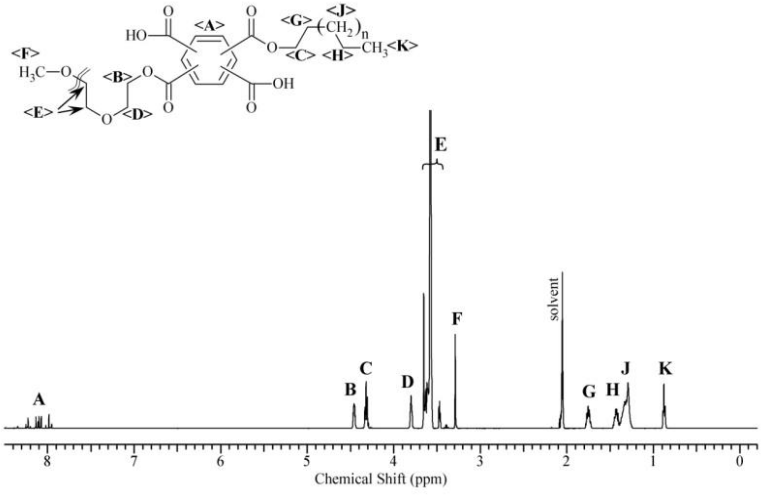


Fig. 7. Spectrum ^{1}H NMR of MPEG550-PMA-Alc (Alc = octyl). 7.90–8.50 (A, 2H); 4.40–4.45 (B, 2H); 4.29–4.33 (C, 2H); 3.80 (D, 2H); 3.45–3.70 (E, 44H); 3,27 (F, 3H); 1.70–1.78 (G, 2H); 1.38–1.47 (H, 2H); 1.27–1.37 (J, 2H); 0.85–0.95 (K, 3H); (n = 4)

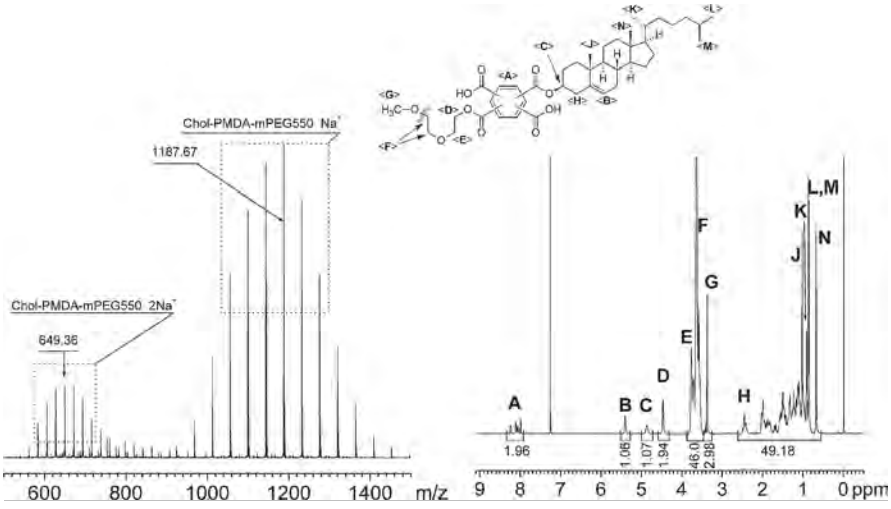


Fig. 8. Mass- and ^{1}H NMR spectrum of MPEG550-PMA-Chol

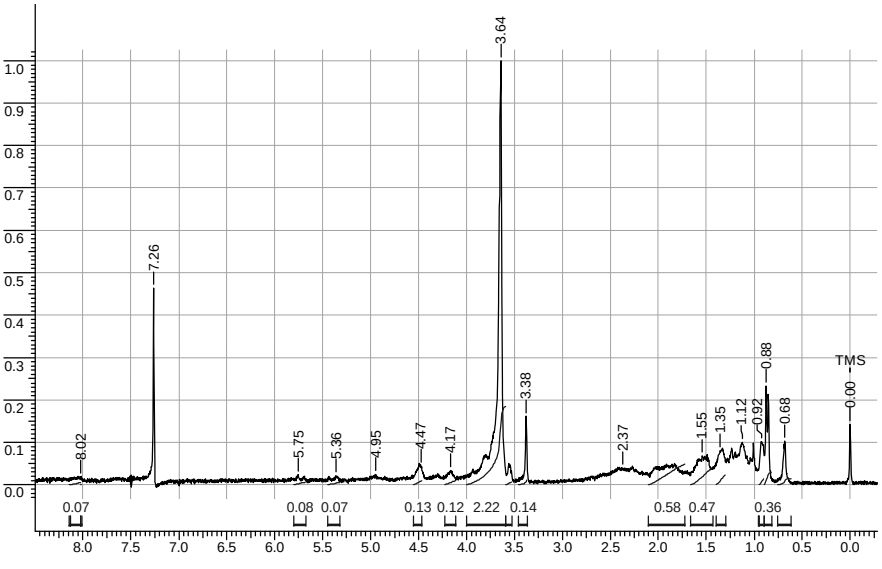


Fig. 9. ^{1}H NMR spectrum of $(MPEG)_2PMA(Chol)_2$

To this end, the acylation reaction of aliphatic alcohols, polyethylene glycols, polyethylene glycol methyl ethers or cholesterol by PMDA can be used for the synthesis of new amphiphilic oligoesters based on pyromellitic acid.

3.3. Surface Activity of OEPA Oligoesters

Amphiphilic behavior and self-assembly of the synthesized oligoesters is expected from having lipophilic alkyl/cholesterol fragments and hydrophilic PEG moieties fragments, simultaneously in the OEPA chemical structure.

Broad surface activity for synthesized OEPAs was targeted to achieve a varying capacity of micelles in the solubilization of lipophilic agents in water. The hydrophilic lipophilic balance (HLB) of OEPAs was calculated using the Griffin formula [29]. The HLB values could be varied between 6.1 and 14.2 by changing the length of the hydrophilic and lipophilic fragments in the oligoester molecule. To confirm the formation of micelles from OEPAs in the aqueous solution, CMC values were determined by using surface tension measurements and the solubilization of pyrene. Molecular weight, values of HLB and CMC of OEPAs are shown in Table 3.

To demonstrate the self-assembling of oligomers in an aqueous medium, the solubilization of pyrene – a common fluorescent probe for monitoring the association behavior of amphiphilic macromolecules was performed [23, 24]. Depending on the environment of pyrene, a bathochromic shift in the spectral band with an enhanced excitation intensity is observed due to the migration of the probe from the hydrophilic phase (water) to the lipophilic phase of the aggregates (micelle cores) [23]. We recorded pyrene excitation spectra in the wavelength range of 300–360 nm over a wide range of the oligoester concentrations in the aqueous solution. The intensity ratio $I_{336.5}/I_{332.5}$ from the excitation spectra plotted against the concentration of the oligoester is shown in Fig. 10. Low $I_{336.5}/I_{332.5}$ intensity ratios calculated for dilute solutions indicate a hydrophilic surrounding of pyrene due to solubilization within the micelle hydrophobic environment, and the transfer of pyrene molecules from water to the micelles. The sharp increase in the intensity ratio corresponds to the critical micelle concentration for each OEPA composition.

Three oligomers based on MPEG550 and different hydrophobic fragments were chosen for CMC measurements using fluorescence probe solubilization. Fig. 10 shows that the CMC values for the oligomers varies between $1 \cdot 10^{-3}$ and $4.8 \cdot 10^{-2}$ mol/l. At the same time fluorescence method gives, in general, lower values for CMC than surface tension measurements, but with the same trend, indicating higher surface activity of more hydrophobic oligomer and lower CMC.

This observation clearly indicates that synthesized oligoesters are able to form micelles in aqueous solution due to the aggregation of single oligomeric molecules in assemblies at different concentration, depending on the HLB (chemical structure) of the oligomers.

The ability to form micelles OEPA and solubilize insoluble substances in water was demonstrated using two non-polar solvents: hexane and benzene, which do not mix with water. At the presence of OEPA micelles, both non-polar solvent molecules quickly migrate into an aqueous phase that can be observed by appearing of turbidity in clear OEPA aqueous solution. Using spectrophotometer, it was demonstrated that hexane and benzene molecules can be physically incorporated within the hydrophobic interior of the OEPA micelle in aqueous solutions in significant quantities (6–8 moles of solvent/mol OEPA).

3.4. Solubilization of Curcumin

Having established the OEPA micelles' ability to solubilize insoluble substances in water, four oligoesters, with different surface activity, were chosen to demonstrate the capability of their micellar solutions to be loaded with the molecules of a lipophilic drug, poorly water-soluble curcumin. All chosen OEPAs demonstrated that curcumin can be successfully solubilized by the OEPA micellar assemblies (Fig. 11). The amount of the solubilized drug differs significantly for different surfactants, showing a general tendency to increase in relation to the increasing length of the hydrophobic fragments in the molecule OEPA.

It was also observed that OEPA micelles stabilize curcumin against decomposition in an aqueous medium. It is known that curcumin is stable at an acidic pH, but is not stable at neutral and alkaline one. In these conditions it decomposes to (4′-hydroxy-3′-methoxyphenyl)-2,4-dioxo-5-hexanal, ferulic acid, and feruloyl methane [30]. It has been demonstrated that more than 90 % of the curcumin rapidly decomposes within 30 min of placement in phosphate buffered saline at pH 7.2.

In this work the stability of curcumin in OEPA micellar aggregates was monitored by UV spectroscopy. Fig. 12 shows that the change in absorbance of curcumin solubilized in micelles OEPA changed slightly for 23 days, confirming high stability of micellar curcumin. Using dynamic light scattering method (DLS), the size distributions of aggregates formed in aqueous solutions of two oligoesters MPEG-PMA-Chol and Chol-PMA-PEG-PMA-Chol were determined (Fig. 13). Small and constant size (50 nm for MPEG-PMA-Chol and 100 nm for Chol-PMA-PEG-PMA-Chol at concentrations 0.003 % and higher) as well as unimodal size distribu-

Table 3

Chemical structure and properties OEPA

OEPA	Lipophilic fragment		M_W PEG or MPEG	Oligoester, M_W calc./found.	HLB	CMC, mmol/l	
MPEG -PMA-ALC	Oct	$-C_8H_{17}$	350	698/715	11,2	13.6*	-
MPEG-PMA-ALC	Oct	$-C_8H_{17}$	550	898/915	12.7	11.7*	0.52**
MPEG-PMA-ALC	Cet	$-C_{16}H_{33}$	550	1010/1027	8.9	2.0*	0.08**
MPEG-PMA-Chol	Chol	$-C_{27}H_{45}O$	550	1154/1188	6.1	0.8*	0.008**
MPEG-PMA-ALC	Oct	$-C_8H_{17}$	750	1098/1115	14.2	11.1*	-
MPEG-PMA-ALC	Cet	$-C_{16}H_{33}$	750	1210/1227	10.4	2.0*	-
Chol-PMA-PEG-PMA-Chol	Chol	$-C_{27}H_{45}O$	600	1808/1778	6.7	-	-
$(MPEG)_2PMA(Chol)_2$	Chol	$-C_{27}H_{45}O$	550	2054/2088	8.2	0.002*	-

Notes: * – determined by using surface tension measurements of aqueous solutions; ** – determined by fluorescence method.

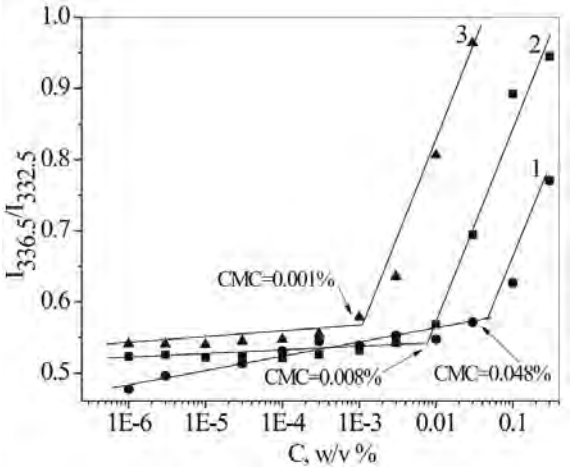


Fig. 10. The intensity ratio $I_{336.5}/I_{332.5}$ of the excitation spectra of pyrene in OEPA solutions vs. OEPA concentration to determine CMC: MPEG550-PMDA-Oct (1); MPEG550-PMDA-Cet (2) and MPEG550-PMDA-Chol (3)

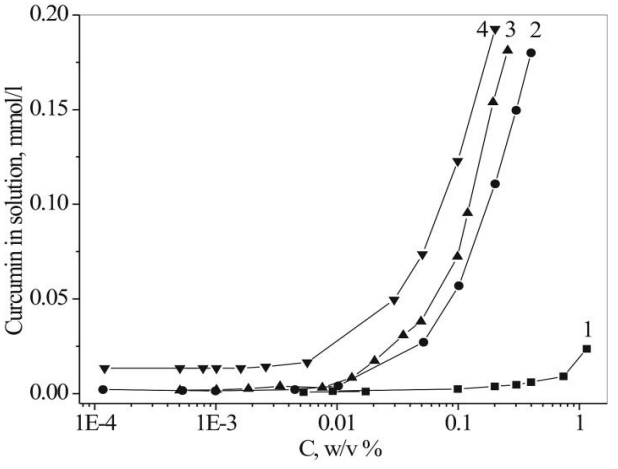


Fig. 11. Dependence of the amount of solubilized curcumin *vs.* OEPA concentration in aqueous solution: MPEG750-PMA-Oct (1); MPEG750-PMA-Cet (2); MPEG550-PMA-Cet (3) and MPEG550-PMA-Chol (4)

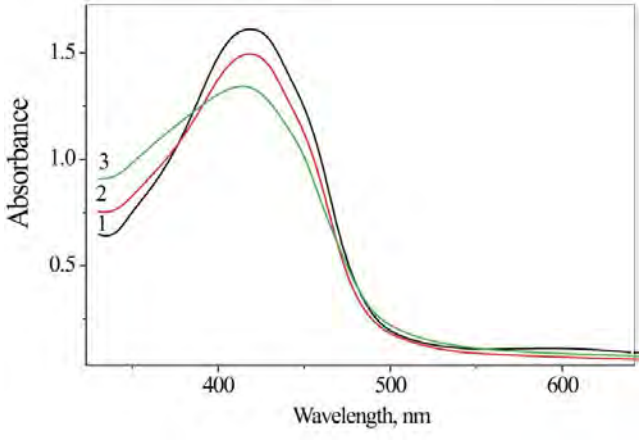


Fig. 12. UV-vis spectra of micellar curcumin-loaded MPEG550-PMA-Cet 0.2 % aq. solution (pH 6.5, 293 K) in 2 days (1); 14 days (2) and 23 days (3)

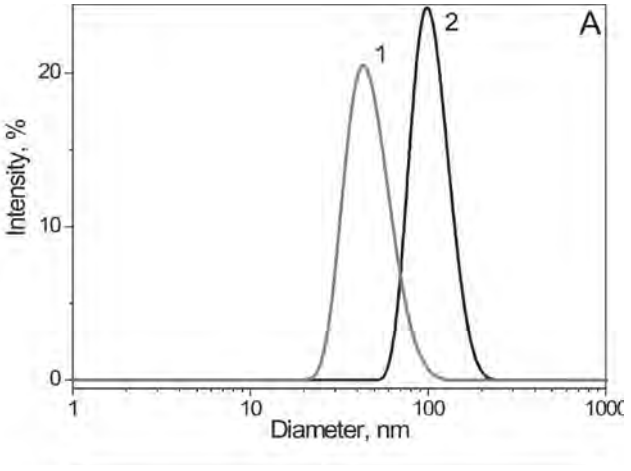


Fig. 13. The size distribution of oligoesteric assemblies in 0.003 wt % aqueous solution: MPEG-PMA-Chol (1) nd Chol-PMA-PEG-PMA-Chol (2)

tion (polydispersity index 1.18–1.2) indicate that assemblies from both oligomers can be considered as nanocarriers for poorly water-soluble substances in different biomedical applications.

Analysis of the ζ-potential shows that the assemblies have a negative surface charge (-57.9 ± 1.9 mV for MPEG-PMA-Chol and -58.2 ± 2.1 mV for Chol-PMA-PEG-PMA-Chol) and can potentially provide greater adhesion to gastrointestinal mucus and more interactions with cellular linings, thus facilitating the bioadhesion between the potential carriers and intestinal epithelial cells [31].

4. Conclusions

Kinetic relativities were determined and the mathematical model was created for the process of PMDA interaction with TBHP. Based on this model the method for synthesis of a new class of amphiphilic oligoesters of pyromellitic acid was developed. Polyethylene glycol or polyethylene glycol mono methyl ethers were used as hydrophilic fragments and primary fatty alcohols or cholesterol – as the lipophilic ones. It is assumed that by facilitating the combination of PEG/MPEG of varying molecular weights with different hydrophobic fragments, the developed approach can be applied to the synthesis of a variety of amphiphilic macromolecules with a regulated surface activity. The structure of the synthesized esters was confirmed by IR- and PMR-spectroscopy. It is shown that the HLB as well as surface activity of oligoesters depend on the length of hydrophilic and hydrophobic fragments and can be adjusted at the process of their synthesis. Above their CMC, the OEPAs solubilize water-insoluble substances, for example such effective antitumor lipophilic drug as curcumin. Additionally, it was demonstrated that loading in OEPA micelles significantly increases the stability of curcumin at neutral pH in aqueous media. The high solubilization capacity of the assemblies and their biodegradability, as well as other properties (size distribution, ζ-potential) make oligoesters promising materials for the design of drug delivery systems.

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СИНТЕЗ ТА СОЛЮБІЛІЗУЮЧІ ВЛАСТИВОСТІ АМФІФІЛЬНИХ ОЛІГОЕСТЕРІВ ПІРОМЕЛІТОВОЇ КИСЛОТИ

Анотація. *Розроблена методика та вперше синтезовано новий клас амфіфільних поверхнево-активних естерів піромелітової кислоти в яких роль гідрофільних фрагментів виконують залишки поліетиленгліколів або монометильованих поліетиленгліколів, а ліпофільних – залишки первинних спиртів*

або холестеролу. Структуру синтезованих естерів підтверджено методами ІЧ та ПМР спектроскопії. При концентраціях більших за ККМ естери здатні солюбілізувати нерозчинні у воді речовини (в тому числі такий ефективний протираковий препарат, як куркумін). Міцелярні нанорозмірні частинки естерів за своїми фізико-хімічними характеристиками (розмірами, хімічною структурою, "ємністю" по відношенню до інкапсульованих препаратів) відповідають вимогам, що пред'являються до систем доставки лікарських засобів і можуть розглядатись як перспективні матеріали для конструювання таких систем.

Ключові слова: *піромелітова кислота, поліетиленгліколь, олігоестер, холестерол, солюбілізація, куркумін.*