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AMPHIPHILIC CHOLESTEROL CONTAINING POLYMERS FOR DRUG DELIVERY SYSTEMS

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Abstract. The interaction of binary copolymers poly(maleic anhydride-*co*-poly(ethylene glycol) methyl ether methacrylate) with cholesterol results in formation of cholesterol containing polymers, which contain from 4.6 to 46.0 mol % monocholesteryl maleic links. Their structure was confirmed using functional analysis and IR spectroscopy. Acidic and anhydride links of these copolymers form polymeric salts if react with alkali. These salts are surfactants which in aqueous medium form hierarchy micelles and micellar aggregates depending on the copolymer concentration. Using conductometry it was found that preferably monomolecular micelles are formed in dilute solutions, and micellar aggregates begin to form at higher concentrations. In aqueous media polymeric salts are able to solubilize such lipophilic substances as Sudan III dye and anticancer drug curcumin. Efficiency of solubilization towards Sudan III grows if the content of monocholesteryl maleic fragment in surfactant increases.

Keywords: amphiphilic polymers, cholesterol, solubilization, drug delivery systems.

1. Introduction

Recently, polymeric colloidal systems have been of great interest as the most promising means for construction of targeted drug delivery systems, particularly for chemotherapy [1-3]. In addition, the polymeric colloidal systems, able to remove toxic substances from the aqueous medium of the human body are of great importance [4]. Promising materials to create such colloidal systems are water-soluble polymers, the solubility of which depends on the structure of macromolecules, pH, temperature, *etc.* These polymers are used to make carrier in the form of micelles [5-7], micro- and nano-capsules, liposomes of different morphology [8-11]. Such polymeric colloidal systems can provide solubilization of highly toxic drugs, minimizing their devastating effects on healthy cells and indirect (side) negative effects, reduce the resistance of cancer cells, increase the efficiency and concentration of drug in tumors. Especial attention is paid to the carriers based on polymers with cholesteryl moieties. The presence of cholesteryl fragments in their macromolecules provides low toxicity, an effective solubilization of water-insoluble drugs and their compatibility with cell membrane plasma. Water-soluble cholesterol containing macromolecules can easily self-assemble to form micelles and aggregates [12-17].

To introduce cholesteryl moieties into amphiphilic polymer, native cholesterol or such monomers as cholesteryl acrylate or cholesteryl methacrylate are often used; polyethylene glycols (PEG) are used as a hydrophilic component. These carriers are characterized by high solubilizing capacity for lipophilic substances, long circulation in the blood stream and slow release of drugs [18-21].

A well-known class of polymers for the creation of drug delivery systems are statistics amphiphilic copolymers with covalently grafted cholesterol fragments and hydrophilic fragments – poly(ascorbyl acrylate, polymetacrylic acid, poly(*N*-isopropylacryl amide) (PNIPAM), polylactide, *etc*. It is shown that in an aqueous medium such macromolecules form micellar colloidal systems or particles with a hydrophobic core and exhibit high loading capacity for lipophilic drugs (ibuprofen, cabazitaxel) [22-23].

Recently we have synthesized a new class of amphiphilic surfactants – triblock esters of pyromellitic acid (MPEG-PMA-Chol) [24] and “Geminy” surfactants (Chol-PMA-PEG-PMA-Chol) (Fig. 1) with hydrophilic fragments of polyethylene glycols or monomethyl ethers of polyethylene glycols and lipophilic – moieties of cholesterol [24, 25].

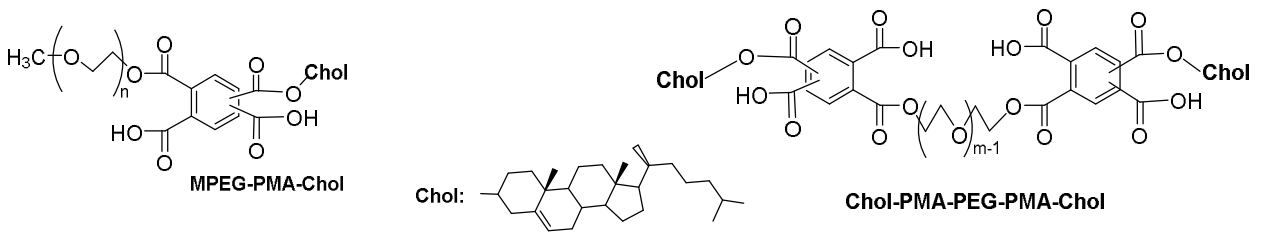


Fig. 1. Chemical structure of cholesterol-bearning amphiphilic surfuctants

In aqueous solutions they form micellar structures which are able to solubilize oleophilic substances, including cholesterol. Such aqueous polymer dispersions are promising as nanocontainers for water insoluble substances to create a new system of drug delivery to the target organ.

Thermally responsive amphiphilic copolymers composed of random PNIPAM copolymer as hydrophilic main chain and hydrophobic grafted side [26] or end-capped [27] cholesteryl fragments have been synthesized. They are able to form in colloidal solutions nanostructures with multiple morphologies including normal spherical shapes, as well as unusual star-like, cubic and cuboids-like shapes, which could be controlled by the formation conditions. The lower critical solution temperature value for both colloidal systems at pH 7.4 was determined to be 311.3 and 306.4 K, respectively. Pyrene as a model hydrophobic compound could be readily encapsulated in these polymeric nanoparticles. Moreover, core-shell nanoparticles are able to encapsulate cyclosporine A and indomethacin. The drug-loading process was analyzed to designate the effect of various parameters on drug encapsulation efficiency. It was shown that cholesteryl grafted polymer yielded the higher encapsulation efficiency for drugs in comparison with end-capped one. The increase in polymer concentration increased drug encapsulation efficiency. Better entrapment was observed for indomethacin compared to cyclosporine A. Indomethacin release from the nanoparticles was responsive to temperature changes, being faster at a temperature around the lower critical solution temperature than below it [28].

Random copolymerization of NIPAM with cholesteryl acrylate results in amphiphilic copolymers, which properties can be tuned by changing the ratio of NIPAM to cholesteryl acrylate [29]. Terpolymer, poly(*N*-isopropylacrylamide-*co*-*N*,*N*-dimethylacrylamide-*co*-10-undecenoic acid) with cholesterol grafted to the hydrophobic segment have been synthesized and folic acid was conjugated to the hydrophilic segment of the polymer through the free amine group for targeting cancer cells that overexpress folate receptors. This polymer also self-assembles to form core/shell nanoparticles that could encapsulate anticancer drug, doxorubicin and exhibit pH-induced temperature sensitivity. These nanoparticles can recognize folate-receptor-expressing cancer cells. Doxorubicin-loaded nanoparticles with folate yield a greater cellular uptake than nanoparticles without folate and, thus, higher cytotoxicity results. These multifunctional polymer core/shell nanoparticles may make a promising carrier to target drugs to cancer cells and release the drug molecules to the cytoplasm inside the cells [30].

A commercial available amphiphilic polymer – cholesterol terminated PEG was used as a novel biomimetic drug delivery system. The PEG block forms the biocompatible micelle coronas and the cholesterol block forms the hydrophobic micelle cores. These biomimetic diblock copolymers were evaluated as nanocapsules for the delivery of hydrophobic drugs [31].

More complete copolymers contain monomeric units of methoxy poly(ethylene glycol) acrylate, 2-hydroxyethyl methacrylate and 2-hydroxyethyl methacrylate – cholesterol conjugates [32].

PEG was conjugated to biodegradable amphiphilic copolymer, poly{(*N*-methyldietheneamine sebacate)-*co*-[(cholesteryl oxocarbonylamido ethyl) methyl bis(ethylene) ammonium bromide] sebacate} (P(MDS-*co*-CES to improve the stability of micelle/DNA complexes in the blood for systemic *in vivo* gene delivery [33]. PEG550–P(MDS-*co*-CES) micelles induced high gene transfection level, comparable to that provided by P(MDS-*co*-CES) micelles. Moreover PEGylated polymers were much less cytotoxic than P(MDS-*co*-CES).

Comb-like amphiphilic copolymers with side cholesteryl moieties have been synthesized on the base of polyallylamine and quaternisation of amino groups was carried out. In aqueous solution they formed nano self-assemblies with a positive zeta potential and were able to encapsulate hydrophobic agents [34]. Another type of comb-like copolymers includes *N*-cholesterol succinyl O-carboxymethyl chitosan [35].

Hydrophobized pullulans with cholesteryl side fragments have been synthesized by Akiyoshi *et al.* [36-40]. In aqueous solutions they form spherical nanoparticles of uniform size. They show no surface

activity up to the concentration 10 times higher than critical concentration of fluorometrically determined aggregation [37]. The nanoparticles have a structure of hydrogel network which is formed by non-covalent cross-linked domains [40]. These nanoparticles are able to form stable complexes with various proteins such as hemoglobin, peroxidase, myoglobin, cytochrome C [36] and α-chymotrypsin [39].

Polyanions containing cholesteryl moieties (Fig. 2) were synthesized *via* random radical copolymerization of sodium 2-(acrylamido)-2-methylpropane-1-sulfonate (AMPS) and cholesteryl 6-methacryloyloxy hexanoate [41, 42]. They contain from 0.5 to 10 mol % of cholesterol, depending on initial ratio of monomers.

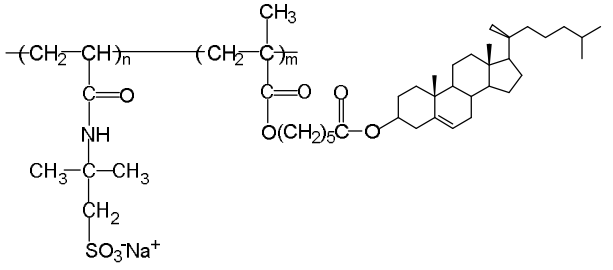


Fig. 2. Chemical structure of cholesterol-bearing polyelectrolyte

Cholesteryl substituted azo-initiator – 4,4-azobis(4-cyano-1-cholesteryl) pentanoate (AzCCP) was prepared [43]. Free radical polymerization of AMPS using AzCPP was performed to obtain a cholesteryl-end-capped polymer (Chol-PAMPS, Fig. 3).

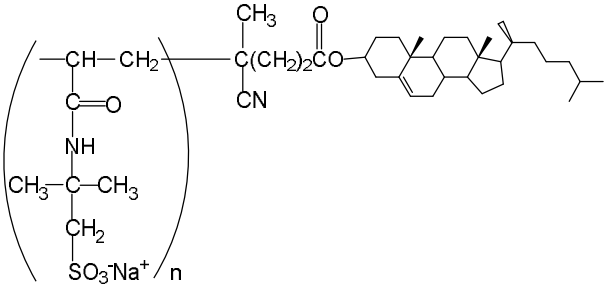


Fig. 3. Chemical structure of cholesterol-end-capped polyelectrolyte

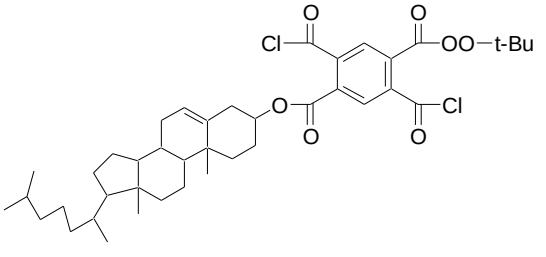


Fig. 4. A multifunctional cholesterol-based peroxide modifier

Recently we synthesized a multifunctional cholesterol-based peroxide modifier with residual acid chloride groups (Fig. 4) [44]. It could be used as a monomer for polycondensation and for the introduction of cholesterol fragments into various polymers.

Water soluble polymers with cholesterol moieties show a strong tendency for self-association in water to form a variety of nanoorganized structures, which may find usefulness as potentials for effective drug delivery systems.

The aim of this work is to develop a new class of amphiphilic polymeric electrolytes containing cholesterol moieties which are able to form micellar solutions with high solubilizing capacity towards lipophilic drugs.

2. Experimental

2.1. Materials

Maleic anhydride (MAN) (Sigma-Aldrich, 95 %); poly(ethylene glycol) methyl ether methacrylate (PEMA) M_n = 246 (Sigma-Aldrich, 98 %); cholesterol (Sigma-Aldrich, 95 %) were dried by azeotropic distillation with benzene. 2,2′-Azobis(2-methylpropionitrile) (AIBN) (Sigma-Aldrich, 98 %); 1,4-dioxane (99,8 %), *N*,*N*-dimethylformamide (DMF) (99.8 %); hexane (99.0 %) and benzene (99.0 %) were purchased from Sigma-Aldrich and purified in accordance with techniques described in [45]. Aniline (99.9 %), Sudan III (analytical standard Sigma-Aldrich); curcumin (Overseal Natural Ingradients Ltd, 99 %) were used without further purification.

2.2. Synthesis of Copolymers

Synthesis of binary copolymers poly(maleic anhydride-co-poly(ethylene glycol) methyl ether methacrylate) (MAn-PEMA). 0.94 g (0.0096 mol) of Man and 0.0141 g ($8.59 \cdot 10^{-5}$ mol) AIBN were loaded to reactor and dissolved under stirring in 9.7 g of 1,4-dioxane ([MAn] – 1.02 mol/l). 2.0 g (0.008 mol) of PEMA was dissolved in 41.3 g of 1,4-dioxane ([PEMA] – 0.2 mol/l) and this solution was added dropwise into the reactor with a speed of 0.02 ml per minute. Mixture was stirred under argon at 343 K for 10 h. 1,4-Dioxane was distilled off and the resulting copolymer MAn-PEMA was triply precipitated by hexane from 1,4-dioxane solution. The content of MAn units in MAn-PEMA copolymer was determined using aniline titrimetry [46]. The synthesized copolymer structure was proved by IR and ^{1}H NMR spectroscopy.

Synthesis of copolymer (cholesteryl maleate-co-maleic anhydride-co-poly(ethylene glycol) methyl ether methacrylate (CholMA-MAn-PEMA) (Fig. 5). Copolymer MAn-PEMA (2.0 g) was dissolved in 16.1 g of DMF in the

round-bottom flask, calculated amount of cholesterol was added and the reaction mixture was stirred under argon blanket at 353 K for 8–10 h. Initial molar ratio of cholesterol units to MAn in MAn-PEMA was varied as: 0.10:1.0; 0.25:1.0 and 0.50:1.0. Conversion of MAn units in copolymer was controlled by aniline titrimetry [46]. After the reaction was finished, DMF was evaporated from the reaction mixture under vacuum, and unreacted cholesterol was extracted with hexane at 333 K. A residue was purified by triple precipitation from solution in 1,4-dioxane with hexane. The composition and structure of CholMA-MAn-PEMA were confirmed by IR and ^{1}H NMR spectroscopy.

Obtaining of sodium salt CholMA-MA-PEMA copolymer (Na-CholMA-MA-PEMA). Na-CholMA-MA-PEMA was obtained by hydrolysis of MAn units at CholMA-MAn-PEMA by 0.1 N aqueous NaOH solution at room temperature at CholMA-MAn-PEMA concentration of 0.1–0.5 wt % (Fig. 7). CholMA-MAn-PEMA was suspended in water and 0.1 N aqueous solution of NaOH was added dropwise until pH 8.0–9.0 was reached.

2.3. Analytical Techniques

UR spectra of the synthesized copolymers were recorded in a thin layer deposited on a potassium bromide tablet, using a Specord-80M infrared Spectrometer, in the range of 400–4000 cm^{-1} with compensation of atmospheric CO_2 and H_2O.

Molecular weight of copolymers was determined by gel-permeation chromatography using Waters Corporation chromatograph with refractive index detector Waters 2410. Tetrahydrofuran was used as an eluent.

To determine *the critical micelle concentration* of the synthesized copolymers (CMC_σ) the surface tension isotherms were performed using a Du Noüy tensiometer at 293 K [47].

Determination of CMC copolymer using conductometric method (CMC_k) [48]. The equivalent conductivity was defined as the ratio of conductivity to the concentration of copolymer.

Characteristic viscosity [η] of copolymers was determined using routine viscometer [46]. Acetone solutions of copolymers were kept for 24 h after preparation to achieve a balance.

The size distribution of copolymer assemblies. The size of colloidal structures (micelles and micellar units) formed by copolymers in the aqueous media was determined by dynamic light scattering method using Particle Sizing Systems Nicomp 380, Inc., Santa Barbara, CA, with laser diode of 15 mW power and photo multiplier detector with system optical geometry of 90 degrees. Aqueous solutions were prepared by dissolving the copolymer in water after Millipore filter (resistance of 18.2 Ohm/m) and further adjusting the pH 7.0 ± 0.05 using a 15% NaOH or HCl aqueous solutions. The measurements were performed the next day after solution preparation.

Solubilization of benzene by Na-CholMA-MA-PEMA copolymers was determined by the method described in [49].

Solubilization of curcumin and Sudan III. A sample of curcumin or Sudan III powder (0.01 g) was mixed with different concentration of Na-CholMA-MA-PEMA solutions at pH 7 or 8, and stirred at room temperature for 48 h. Excessive dye was filtered off using paper filter. The UV-Vis spectra of die-solubilized copolymer solutions were recorded using Varian Cary 5000 at 293 K. The concentration of solubilized dye was determined from the absorption spectra at characteristic wavelength of 470 nm for curcumin and 540 nm for Sudan III, using a calibration curve [49].

3. Results and Discussion

Binary copolymer MAn-PEMA was obtained by radical copolymerization of MAn with PEMA initiated by AIBN in 1,4-dioxane solution. It is known that MAn is capable to form homopolymer in solution [50-52]. In order to escape a formation of PEMA homopolymer, a multiple excess of MAn was used. For this goal the solution of PEMA in 1,4-dioxane was slowly added by drops to MAn solution. Resulting copolymer consists of MAn (46 %) and PEMA (54 %) molar unitsю This structure is similar to alternating MAn-PEMA copolymer.

Acylation of cholesterol by MAn-PEMA copolymer results in CholMA-MAn-PEMA triple copolymer. The reaction occurs through the interaction of cholesterol hydroxyl group with MAn links in copolymer (Fig. 5). The content of cholesteryl maleic (CholMA) links in copolymer depends on the initial ratio of reagents and varies from 4.6 to 46 mol %.

The values of intrinsic viscosity of starting binary copolymer MAn-PEMA and triple copolymers CholMA-MAn-PEMA with different content of CholMA links in acetone are in the range of 0.09–0.11 (Table 1), which is typical for oligomers. This was confirmed by gel-chromatographic determination of their molecular weights (Table 1).

Composition of copolymers MAn-PEMA and CholMA-MAn-PEMA was determined using aniline titrimetry (Table 1) and IR spectroscopy. IR spectrum shows the absorption bands, confirming the structure of the MAn-PEMA and CholMA-MAn-PEMA copolymers (Fig. 6a). In particular, the absorption bands at 1788 and 1852 cm^{-1} in the MAn-PEMA spectra correspond to ν(C=O) in MAn units, the band at 1732 cm^{-1} – to ν(C=O), at 1248 cm^{-1} – to ν(C–O) in ester fragments within links PEMA and at 1108 cm^{-1} – to ν(C–O) at oxyethyl substituent in PEMA. Valence and deformation vibrations of CH groups appear at 2980–2880 and 1456–1350 cm^{-1}, respectively.

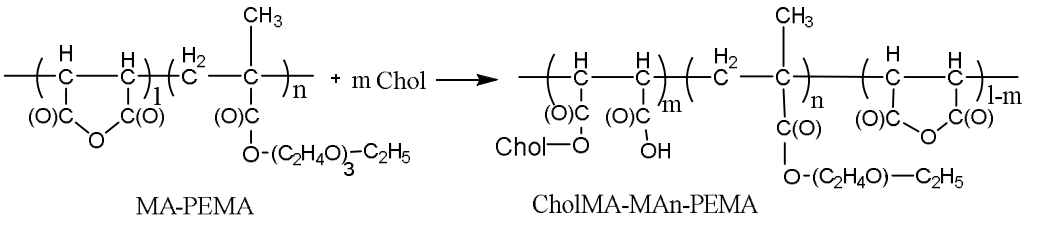
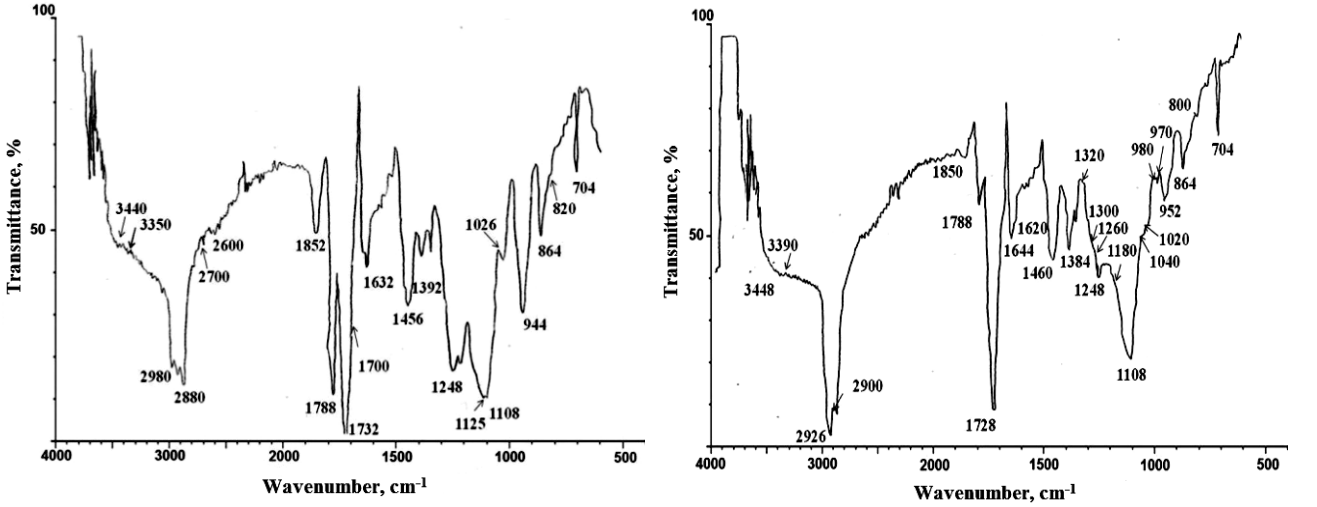


Fig. 5. The scheme of CholMA-MAn-PEMA triple copolymer formation

Table 1

The composition and intrinsic viscosity [η] of CholMA-MAn-PEMA copolymers

Content of links, mol %			[η] in acetone	Molecular weight	
CholMA	MAn	PEMA		M_w	M_n
–	46.0	54.0	0.090	–	–
4.6	41.4	54.0	0.113		
11.5	34.5	54.0	0.100	7000	5000
23.0	23.0	54.0	0.104	10000	6500
46.0	–	54.0	0.091		



a) b)

Fig. 6. IR spectra of copolymers: MAn-PEMA (a) and CholMA-MAn-PEMA (b).
Content (mol %) of CholMA 23.0, MAn 23.0 and PEMA 54.0

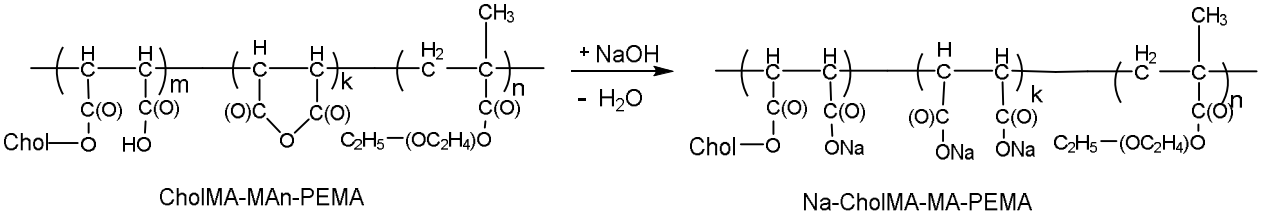


Fig. 7. The formation of Na-CholMA-MA-PEMA by alkali hydrolysis
of MAn fragments in CholMA-MAn-PEMA molecules

In a spectrum of CholMA-MAn-PEMA triple copolymer (Fig. 6b) a sharp decrease in the intensity of MAn absorption bands at 1852 and 1788 cm^{-1} ν(C= O) is observed. This indicates the decreasing content of MAn links in copolymer caused by their esterification or hydrolysis. The absorption band of ν(O–H) at 3200–2500 cm^{-1} and broad absorption band of δ(O–H) at 948–930 cm^{-1} also confirm the formation of carboxyl groups as a result of cholesterol acylation (Fig. 5). Simultaneously we observe the appearance of the absorption bands at 970, 980, 800 and 1040 cm^{-1}, which belong to cholesterol fragments. It should be noted that the increase in cholesteryl links content from 4.6 to 23.0 mol % results in increasing intensity of the band at 1040 cm^{-1} to confirm the proposed CholMA-MAn-PEMA structure.

In order to impart amphiphilic properties to CholMA-MAn-PEMA triple copolymer and alkali hydrolysis of MAn fragments was carried out in accordance with the Scheme (Fig. 7).

The resulting copolymer Na-CholMA-MA-PEMA possesses amphiphilic properties due to the presence of hydrophilic carboxylate groups and polyoxyethylene substituents, as well as lipophilic cholesteryl fragments.

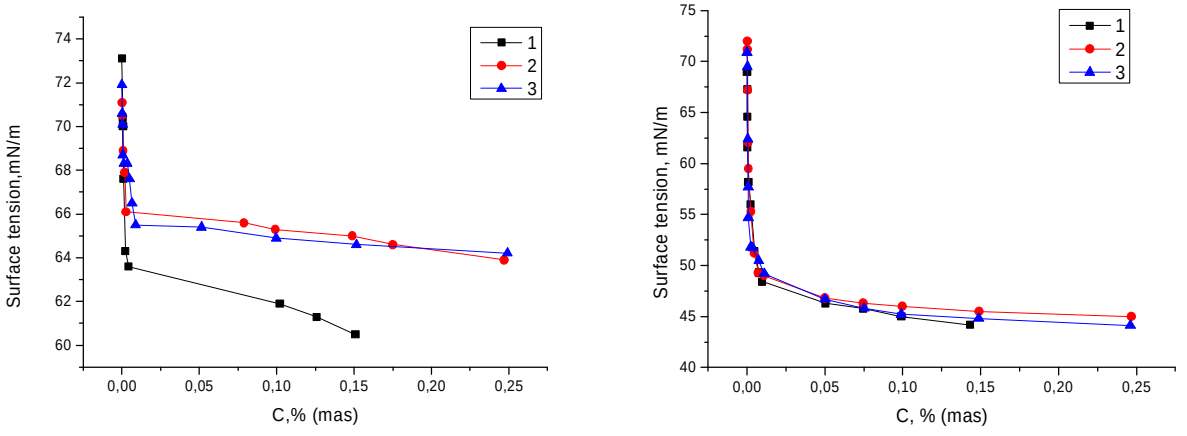
3.1. Colloidal Properties of Obtained Amphiphilic Copolymers

It was shown that Na-CholMA-MA-PEMA copolymers are able to form colloidal systems in aqueous medium. They reduce the surface tension at the interface water-air to 66–46 mN/m, depending on pH and the content of CholMA links (Fig. 8). At pH 7.0 the increase of lipophilic CholMA links content in compound increases the copolymer surface activity (Fig. 8a). If pH value of the aqueous solution decreases from 7.0 to 4.0, the surface activity increases (Fig. 8a, b). At pH 4.0 the surface activity of copolymer does not depend on the content of CholMA links, probably due to higher total lipophility of copolymer.

The presence of ionized carboxyl groups in Na-CholMA-MA-PEMA macromolecules allows to investigate the formation of colloidal structures using conductometric method. The dependence of equivalent conductivity of aqueous colloidal solutions *via* the square root of the Na-CholMA-MA-PEMA concentration is characterized by the sharp break at certain concentration (Fig. 9).

At Na-CholMA-MA-PEMA concentrations prior to the breaking point, the equivalent conductivity sharply increases with dilution. This fact can be explained by the decrease in association of carboxylate groups in Na-CholMA-MA-PEMA macromolecule with low molecular ions and increase of their mobility [53]. The copolymer concentration under which a break on the curve was observed is the beginning of self-organized micelle formation and can be designated as CMC_k.

Table 2 shows that the values of CMC_k are lower than CMC_σ which correspond to the point of break on the surface tension isotherm. CMC_k corresponds to the concentration under which the formation of micellar structures starts and CMC_σ is determined from isotherms of surface tension (a concentration of adsorbed layer saturation at the water-air interface by Na-CholMA-MA-PEMA and its aggregation process has completed) [54]. Obviously, this saturation will occur at higher concentrations than CMC_k ones. This is confirmed by the fact that the size of Na-CholMA-MA-PEMA colloidal particles at CMC_k concentrations determined by dynamic light scattering is 1–4 nm. It allows to qualify such micelles as "unimeric". The size of micellar aggregates at CMC_σ is much greater. However, medium-size of these aggregates reduces with the increase in CholMA links content in the copolymer (Table 2), which is obviously due to the increased hydrophobic interaction of cholesterol fragments in micellar aggregates.



a) b)

Fig. 8. Surface tension isotherms of Na-CholMA-MA-PEMA water solutions at: pH 7.0 (a) and pH 4.0 (b). Content of CholMA links (mol %) in copolymer: 23.0 (1); 11.5 (2) and 4.6 (3)

Table 2

Size of micelles and micellar aggregates formed by Na-CholMA-MA-PEMA in aqueous medium at CMC_k and CMC_σ. critical concentration of solubilization (*CCS*)

Content of links, mol %			*$CMC_k \cdot 10^4$, %	Size of micelle at CMC_k, nm	**$CMC_\sigma \cdot 10^4$, %	Size of micellar aggregates at CMC_σ, nm	$CCS \cdot 10^4$, %	
CholMA	MA	PEMA					pH 7.0	pH 8.0
4.6	41.4	54.0	1.6	1	11.0	120	4.4	10.6
11.5	34.5	54.0	1.8	1	16.0	80	5.9	12.6
23.0	23.0	54.0	2.3	4	15.0	50	8.3	12.4
46.0	–	54.0	2.6	4	–	–	–	24.8

Notes: * CMC_k determined at pH 7.0 by conductometric method; ** CMC_σ determined from surface tension isotherms at pH 7.0.

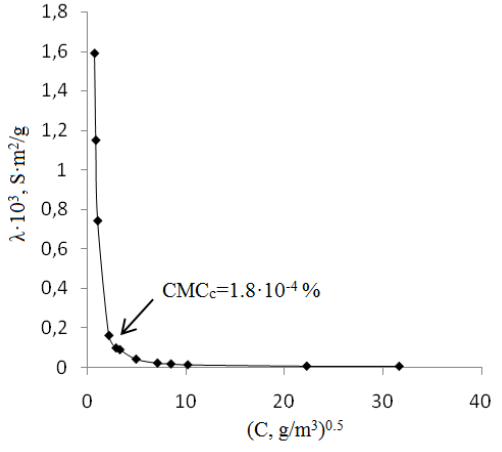


Fig. 9. Dependence of the equivalent conductivity of colloidal solutions of Na-CholMA-MA-PEMA *via* the root of the concentration at pH 7.0. Content (mol %) of CholMA units – 11.5, MA – 34.5 and PEMA – 54. CMC_c = $1.8 \cdot 10^{-4}$ %

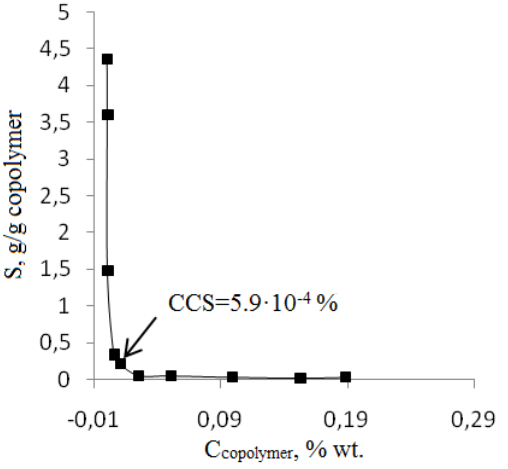


Fig. 10. An efficiency of Sudan III solubilization in Na-CholMA-MA-PEMA aqueous colloidal solutions *via* concentration of Na-CholMA-MA-PEMA. The content of the links in copolymer (mol %): CholMA – 11.5; MA – 34.5 and PEMA – 54; pH 7

3.2. Solubilization of Lipophilic Substances

It was expected that micelles and micellar aggregates of Na-CholMA-MA-PEMA triple copolymers which have lipophilic core formed by cholesterol fragments will be able to solubilize lipophilic substances such as dye Sudan III, anticancer drug curcumin, hydrocarbons.

Typical dependence of solubilization efficiency (expressed as g of solubilized Sudan III per g of copolymer) in Na-CholMA-MA-PEMA aqueous colloidal solutions *via* concentration of Na-CholMA-MA-PEMA is shown in Fig. 10. Solubilization significantly increases if concentration of copolymer in solution tents to zero. At the concentrations higher than CMC_σ ones the efficiency of solubilization is poor and practically does not depend on copolymer concentration.

It can be seen from Fig. 10 that at the same Na-CholMA-MA-PEMA concentrations the solubilization of Sudan III increases with the increase of lipophilic moieties CholMA content in copolymer. This fact can be explained by growth of lipophilic core volume at micelles and micellar aggregates.

The efficiency of solubilization at the concentrations lower than CMC_σ is significantly greater at pH 8.0 than that at pH 7.0. The reason is the increase of micelles electrostatic stabilization at high pH, due to a greater degree of ionization and solvation of carboxyl groups in hydrophilic moieties of surfactant. It is known that the increase of electrolyte concentration in an aqueous solution of surfactant increases solubilization of Sudan III [55]. The authors suggest that screening of polar groups at surfactant molecules increases critical parameter of the package, resulting in a transformation of spherical micelles into elongated form and in increasing both the

number of aggregation and the volume of hydrophobic core of the micelles. Confirmation of this conclusion is the absence of Sudan III solubilization at pH 4.0 when carboxyl groups are not ionized.

At the concentrations of Na-CholMA-MA-PEMA lower than CMC_k concentrations, copolymer molecules generally exist as "unimer" micelles. Lipophilic core of "unimer" micelles is forming through hydrophobic interactions of cholesterol fragments at surfactant molecule and is capable to solubilize Sudan III. The increase of Na-CholMA-MA-PEMA concentration from CMC_k to CMC_σ results in aggregation of "unimer" micelles and formation of ordered structures with less accessibility of lipophilic cores and less capability of Sudan III solubilization.

The concentration of surfactant at point of brake on the curve (Fig. 11) is a critical concentration of solubilization (*CCS*) and could be used for determination of *CMC* for low molecular weight surfactants [55]. *CCS* is close to CMC_σ, determined from surface tension isotherms (Table 2).

It is shown that efficiency of solubilization depends on the nature of solubilized molecules. For example, Sudan III and curcumin are solubilized better than benzene (Fig. 12). Obviously, this is due to the better solubility of benzene in water in comparison with Sudan III and curcumin.

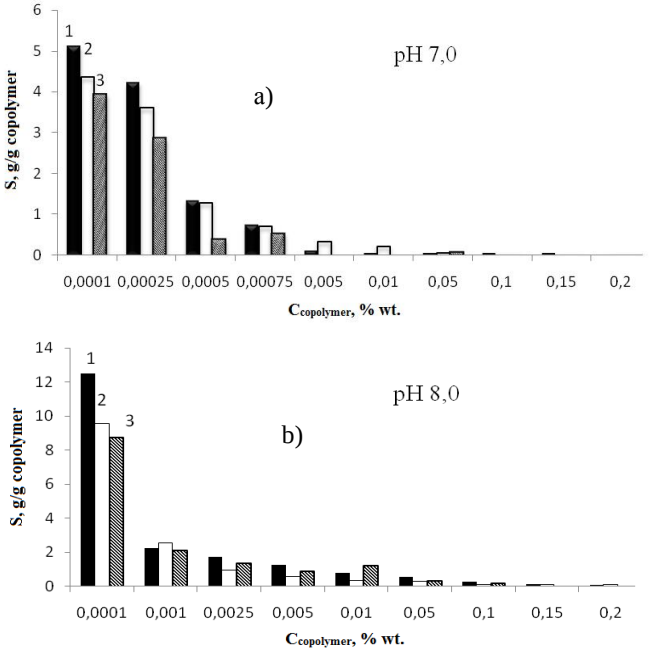


Fig. 11. Efficiency of Sudan III solubilization in Na-CholMA-MA-PEMA aqueous colloidal solutions at different concentrations and compositions of surfactants: pH 7.0 (a) and pH 8.0 (b). Content (mol %): CholMA – 23, MA – 23, PEMA – 54 (1); CholMA – 11.5, MA – 34.5, PEMA – 54 (2) and CholMA – 4.6, MA – 41.4, PEMA – 54 (3)

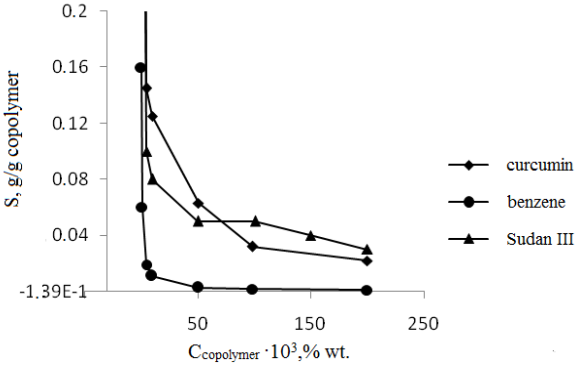


Fig. 12. Efficiency of copolymer solubilization *via* concentration of surfactant. The content of the links in Na-CholMA-MA-PEMA molecule (mol %): CholMA – 23.0; MA – 23.0 and PEMA – 54.0

4. Conclusions

The interaction of binary regular copolymer poly(maleic anhydride-*co*-poly(ethylene glycol) methyl ether methacrylate) with cholesterol results in formation of monocholesteryl maleic links in copolymer within 4.6–46.0 mol %. In the presence of sodium alkali the copolymers form sodium salts, which are typical amphiphilic surfactants. They are able to reduce surface tension of water to 45 mN/m and form colloidal solutions. Formation of monomolecular micelles in water solutions was established by conductometry. Critical concentrations of aggregation were determined from surface tension isotherms. It was shown that in aqueous media at pH 7.0 colloidal particles of surfactants are capable to solubilize such lipophilic substances as benzene, Sudan III dye and anticancer drug curcumin. Solubilizing capacity of aggregates towards Sudan III grows if the content of monocholesteryl maleic fragment in surfactant increases. Adding 1-octanol results in release of solubilized Sudan III to oleofilic phase.

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АМФІФІЛЬНІ ХОЛЕСТЕРОЛОВМІСНІ ПОЛІМЕРИ ДЛЯ СИСТЕМ ДОСТАВКИ ЛІКАРСЬКИХ ЗАСОБІВ

Анотація. *Взаємодією бінарного кополімеру полі(малеїновий ангідрид-ко-поліетиленгліколь метакрилат) з холестеролом одержані нові холестероловмісні кополімери, що містять від 4.6 до 46 % мол. монохолестерилмалеїнатних ланок. Їх структуру підтверджено функціональним аналізом, а також ІЧ спектроскопією. При взаємодії з лугом кислотні та ангідридні ланки кополімерів утворюють солі. Ці солеподібні кополімери є поверхнево-активними речовинами, які у водному середовищі утворюють ієрархію міцел та міцелярних агрегатів в залежності від концентрації кополімера. Методом кондуктометрії встановлено, що у розведених розчинах утворюються, переважно мономолекулярні міцели, а при більших концентраціях починають формуватись міцелярні агрегати. Солеподібні кополімери здатні солюбілізувати у водному середовищі ліпофільні речовини, зокрема, барвник Судан ІІІ та протираковий препарат куркумін. Показано, що ефективність солюбілізації Судану ІІІ зростає симбатно вмісту холестерилмалеїнатного фрагменту у кополімері.*

Ключові слова: *амфіфільні полімери, холестерол, солюбілізація, засоби доставки лікарських засобів.*