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Self-assembly of cationic polymers in mixed polymer-lipid monolayers at the liquid-air interface

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Abstract

Purpose: The self-assembly of cationic polymers at the liquid-air interface is the most important process for the design of new targeted drug delivery systems, including lipid-polymer hybrid nanoparticles.

We studied the self-assembly of cationic pH-sensitive polymers at the liquid-air interface in mixed Langmuir polymer-lipid monolayers of cholesterol with Lipoid S100.

Experimental: We used synthetic approaches to the synthesis of ionene polymer and studied its physicochemical properties by NMR, IR, HPLC, and gel permeation chromatography. The surface behavior and states of polymer monolayers and their mixtures with lipids (compressibility and molecular area) at the liquid-air interface were examined using the Langmuir-Blodgett technique. The resulting lipoplexes were studied by dynamic light scattering (average weight diameter and zeta potential).

Conclusions: High stability of lipid-polymer nanoparticles was achieved for compositions of mixed monolayers with the compressibility modulus (C_s^{-1}) of at least 50 mN/m. In this case, the particle sizes were in the range from 32 to 73 nm and the zeta potential values for non-quaternized cationic polymers were strongly negative (from –15 to –45 mV), while for the ionene polymers they were significantly positive (from +8 to +49 mV).

Keywords: Self-assembly at interfaces, Polymer-lipid nanoparticles, Langmuir monolayers, Ionene polymers

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1. Introduction

Self-assembly at the liquid-air interface plays a crucial role in designing new systems for targeted drug delivery. Lipid-polymer hybrid nanoparticles (lipoplexes), which combine the advantages of liposomes and polymerosomes and compensate for their disadvantages, are of greatest interest for the delivery of various drugs, including nucleic acids [1, 2].

The important requirement for polymers chosen as platforms is not only their low toxicity, amphiphilicity, ability to form micelles, Langmuir monolayers, and thin films at various interfaces, but also their cationic charge. Cationic polymers, especially those containing ionene units with a high positive charge, combined with lipids, are especially attractive for developing lipid-polymer nano-systems at the liquid-air interface enhancing the efficiency of drug delivery to the cell nucleus. Several studies have shown that cationic pH-sensitive methyl methacrylate copolymers (Eudragit) when combined with lipids, such as cholesterol, lecithin or their derivatives, effectively deliver nucleic acids to target cells with minimal cytotoxicity via polymeric nanoparticles [3, 4, 5]. Controlled incorporation of ionene chains of a given length into methacrylic amides or esters, such as methoxy[oligo(propylene glycol)-co-oligo(ethylene glycol)-methacrylate-co-N-[3-(dimethylamino)propyl]methacrylamide)

and their methyl iodide-quaternized analogs, is of particular interest not only due to their strong bactericidal, antioxidant, and fungicidal properties but also due to their ability to self-assemble at interfaces. All polymers were found to form stable mixed monolayers with lipids (cholesterol, dihexadecyl phosphate) at various interfaces.

Ionene polymers containing ammonium groups represent a unique class of cationic polymers since they are characterized by a very high density of their ionic sites and controllable charge densities along their skeletal chains [6-9]. In addition, the synthesis of ionene polymers is usually relatively simple.

In this work, we investigated the self-assembly at the water-air interface of three cationic pH-sensitive polymers (Fig. 1): 1) methyl methacrylate-butyl methacrylate-dimethylaminoethyl methacrylate (MMA-BMA-DMAEM), similar to Eudragit 100; 2) ionene macromonomer – PK-Im; 3) homopolymer ionene macromonomer PK-Im – PK-Ip, each combined with a mixture of lipids. The study focused on: 1) the surface behavior of the polymers in mixed polymer-lipid monolayers composed of cholesterol and Lipoid S100 as model lipids; 2) the colloidal-chemical properties of hybrid polymer-lipid nanoparticles produced by precipitation.

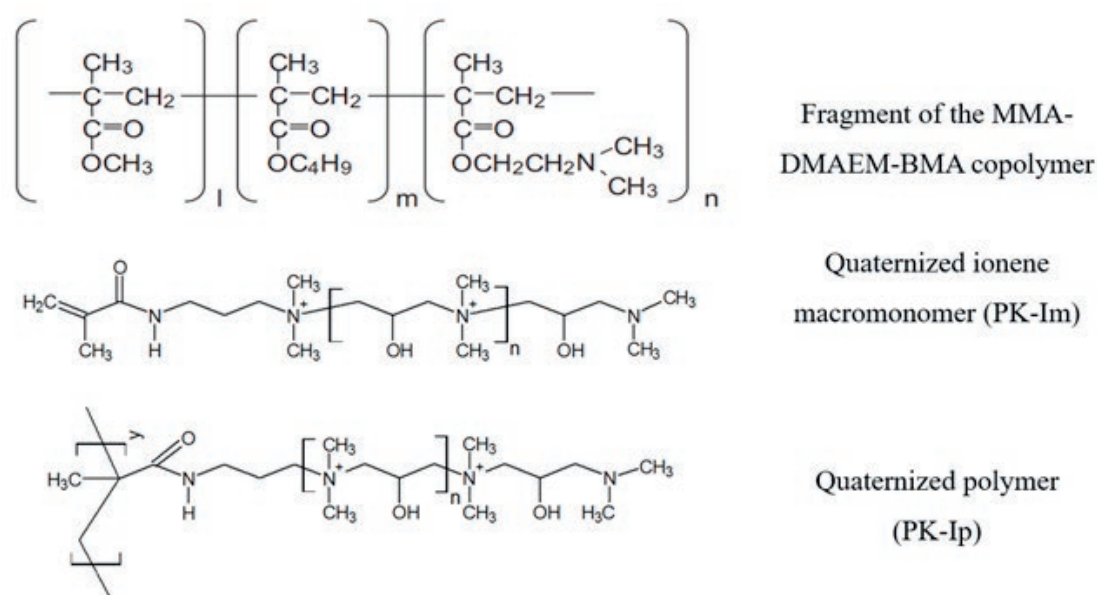


Fig. 1. General formulas of cationic pH-sensitive polymers

2. Experimental

2.1. Materials and methods

Dimethylaminopropyl methacrylamide (DMAPMA) and 1,3-bisdimethylaminopropan-2-ol (BDAP) were purchased from Sigma-Aldrich. Epichlorohydrin (ECH) and initiator 2,2'-azobisisobutyronitrile (AIBN) were purchased from Acros Organics.

Solvents, i.e. acetone, hexane, chloroform, methanol, and DMSO, were purchased from Acros Organics. Deionized water was produced using a Chromatek deionizer, Russia, Dzerzhinsk, with an electrical conductivity of less than 0.1 $\mu\text{Ohm/cm}$. A statistical copolymer (MMA-BMA-DMAEM) with MW = 89,000 was obtained by radical polymerization in toluene at 60 °C using AIBN as the initiator. The reaction proceeded to high conversion, with a monomer feed ratio of 1:1:2.

2.2. Synthesis of PK-Im macromonomer

The macromonomer was synthesized by polycondensation of epichlorohydrin (ECH) and 1,3-bisdimethylaminopropan-2-ol (BDAP) in the presence of dimethylaminopropyl methacrylamide (DMAPMA) in water with stirring at 25 °C in a reactor with a reflux condenser. Epichlorohydrin was added to a mixture of amines in water under stirring and cooling for 30 min, then the reaction mixture was kept at room temperature. The ratio of ECH:BDAP:DMAPMA was 3.5:3:1 with a total reagent content of 40% by weight. After 6 hours of synthesis, the conversion of the initial monomer was 60%, and after 24 hours it reached 74%. The macromonomer was precipitated with acetone. IR spectrum (Shimadzu, Japan) was as follows: in the region of O-H stretching vibrations it was 3,500–3,400 cm^{-1} (broad and intense band), in the region of carbonyl group stretching vibrations it was 1,730–1,668 cm^{-1} , and in the region of C-H bond stretching vibrations of the hydrocarbon skeleton it was 3,000–2,800 cm^{-1} . The ^1H NMR spectrum of the ionic macromonomer (Agilent DD2 NMR 400WB, USA; tetramethylsilane standard, DMSO- D_6 solvent) showed the following signals (δ , ppm): $-\text{CH}_3$ in different positions designated by letters at 1.7 (m), 2.49 (q), and 3.25–3.26 (d, e); the vinyl group $=\text{CH}_2$ (a) at 5.25–5.32 and 5.52–5.75; $-\text{CH}_2-$ at 3.18–3.25 (g, h) and 3.48–3.52 (c, d); and $>\text{CH}-$ at 4.16–4.20. The molecular weight of the macromonomer (^1H NMR) was 642 g/mol,

which corresponded to 4 atoms of quaternary ammonium in the monomer.

2.3. Synthesis of PK-Im ionene polymer

Polymerization of PK-Im ionene macromonomer was carried out in a stirred reactor in a nitrogen flow in DMSO at a monomer concentration of 20% by weight. The reaction was carried out at 70 °C with 1% by weight of AIBN as the initiator, achieving a conversion of 89.9%. The resulting polymer was precipitated in acetonitrile. The homopolymer had $M_w = 13,900$, $M_n = 6,300$, and polydispersity = 2.2 (HPLC chromatograph Chromos ZhK-301, Russia; column Agilent PL aguagel-OH, aqueous eluent with 0.1 M Na_2SO_4 , polyethylene glycol standards).

2.4. Study of monolayers in the aqueous subphase

Polymer self-assembly into monolayers was studied by recording surface pressure isotherms $\pi = f(A)$ using a Wilhelmy plate method with a KSVmini device (Finland). Deionized water was used as an aqueous subphase. The polymer with a concentration of 1 mg/ml (10 μl) and lipid solutions (Lipoid S100 or cholesterol) in chloroform with a concentration of 1 mg/ml (5–20 μl) were added dropwise to the aqueous subphase using a chromatographic syringe. Each new portion was added after the previous one had completely spread. The introduction of portions “drop by drop” promotes the formation of stable Langmuir monolayers. The solvent evaporation time was 30 minutes, the monolayer compression rate was 10 mm/min. The electrical conductivity of deionized water (Chromatek deionizer, Russia) was less than 0.1 $\mu\text{Ohm/cm}$. To prepare mixed polymer-lipid monolayers, lipid was first applied to the surface of the aqueous subphase and after 30-minute evaporation of the solvent, the polymer solution was applied dropwise.

2.5. Lipoplex studies

Zeta potential was determined by dynamic light scattering (DLS) on a NanobrookOmni instrument (Brookhaven Instruments Corporation, USA). For analysis, 1 mg of the sample was dispersed in 25 ml of deionized water (or a buffer solution with pH = 7.4), ultrasonicated for 5 min, and filtered through a polyethersulfone filter with a pore size of 0.8 μm . Average hydrodynamic diameter

was obtained by using polymodal analysis of the correlation function (25 ± 0.1 °C, angle 90 ° in the range from 0.1 to 5,000 nm in 1 cm polystyrene cuvettes).

2.6. Methods for the preparation of nanoparticles with an ionene homopolymer

Weighed portions of Lipoid S100 and cholesterol were placed in a round-bottomed flask and dissolved in 10 ml of chloroform. The solvent was removed on a rotary evaporator for 1 hour. Then the sample was left in air for 10 minutes to remove residual solvent. A weighed portion of an ionene polymer was dissolved in 10 ml of water. The lipid film was hydrated with the resulting solution. The resulting suspension was treated in an ultrasonic bath for 30 min.

3. Results and discussion

3.1. Mixed cholesterol and Lipoid S100 Langmuir monolayers

Fig. 2 shows the compression isotherms of mixed lipid monolayers on pure water at different molar ratios. The molecular area A_0 (the area occupied by one lipid molecule in a monolayer) for Lipoid S100 was 1.4 nm^2 . This differed significantly from the value for egg lecithin (1-oleoyl-2-palmitoyl-phosphatidylcholine), $0.56 \pm 0.2 \text{ nm}^2$ [10]. The molecular area of cholesterol was 0.38 nm^2 , which was almost identical to the results of previous studies [11].

The elasticity of the resulting lipid film, a potential shell for lipoplexes, and its mechanical properties were calculated using the compressibility modulus (C_s^{-1}) by the formula:

$$C_s^{-1} = -A_0(d\pi/dA)_{p,T} \quad (1),$$

where A_0 is the area occupied by one molecule in a monolayer at a surface pressure of π and $d\pi/dA$ is the gradient or rate of changes in the surface pressure during the spread of the surface film. The compressibility modulus of cholesterol on the subphase of pure water with pH 5.0–6.0 is about 700 mN/m [11], however, the analysis of the obtained data showed that cholesterol monolayers can be unstable at the “water-air” interface and can have two phase states upon compression: the first at about $\pi = 10 \text{ mN/m}$ and the second at about $\pi = 30 \text{ mN/m}$ on the aqueous subphase under the analyzed conditions (compression rate,

pH, and the temperature of the experiment). The compression conditions for the monolayers were selected in such a way as to make it possible to obtain mixed polymer-lipid films in the future. Fig. 2 demonstrates improved elasticity of mixed lipid monolayers as the Lipoid S100 proportion increases: the maximum compressibility modulus (elasticity) increases in the following series of Lipoid S100 to cholesterol ratios: $1:1 < 3:1 < 5:1 < 7:1$.

Thus, based on the study of elasticity (by compressibility modulus) of lipid monolayers, it follows that the basic lipid support can be a mixture of non-ionizing lipid cholesterol with ionizing phosphatidylcholine-containing lipids (conventionally called “lecithin”), while the main requirement for a stable phase state of the monolayers is Lipoid S100 : cholesterol ratio of 5:1 or 7:1.

3.2. Study of cationic polymers self-assembly in homogeneous and mixed polymer-lipid Langmuir monolayers

The primary and essential requirement for the formation of hybrid polymer-lipid nanoparticles is the formation of stable mixed “polymer-lipid” monolayers at the “water-air” interface.

The state of the surface film of the copolymer (MMA-BMA-DMAEM) depended on the conditions of its formation (volume of the applied polymer solution, compression rate). The molecular area changed from 260 nm^2 to 180 nm^2 with increasing copolymer concentration, while the compressibility modulus remained unchanged at about 45 mN/m, which is characteristic of liquid-condensed films whose elasticity is close to that of lecithin's. Fig. 3 shows a typical compression isotherm and the compressibility modulus of the polymer (MMA-BMA-DMAEM) monolayer on water at the maximum copolymer concentration.

In contrast, PK-Im and PK-Ip ionene cationic polymers did not form stable monolayers on the water surface. However, all studied polymers formed stable mixed Langmuir monolayers with cholesterol and Lipoid S100. Fig. 4 shows the compression isotherms and hysteresis curves of mixed monolayers of ionene polymers and Lipoid S100 (a, c) and cholesterol (b, d).

The degree of hysteresis was estimated by the formula $\Delta A_0 = A_0 - A'_0$, where A'_0 is the limiting area of the lipid under tension in the mixed

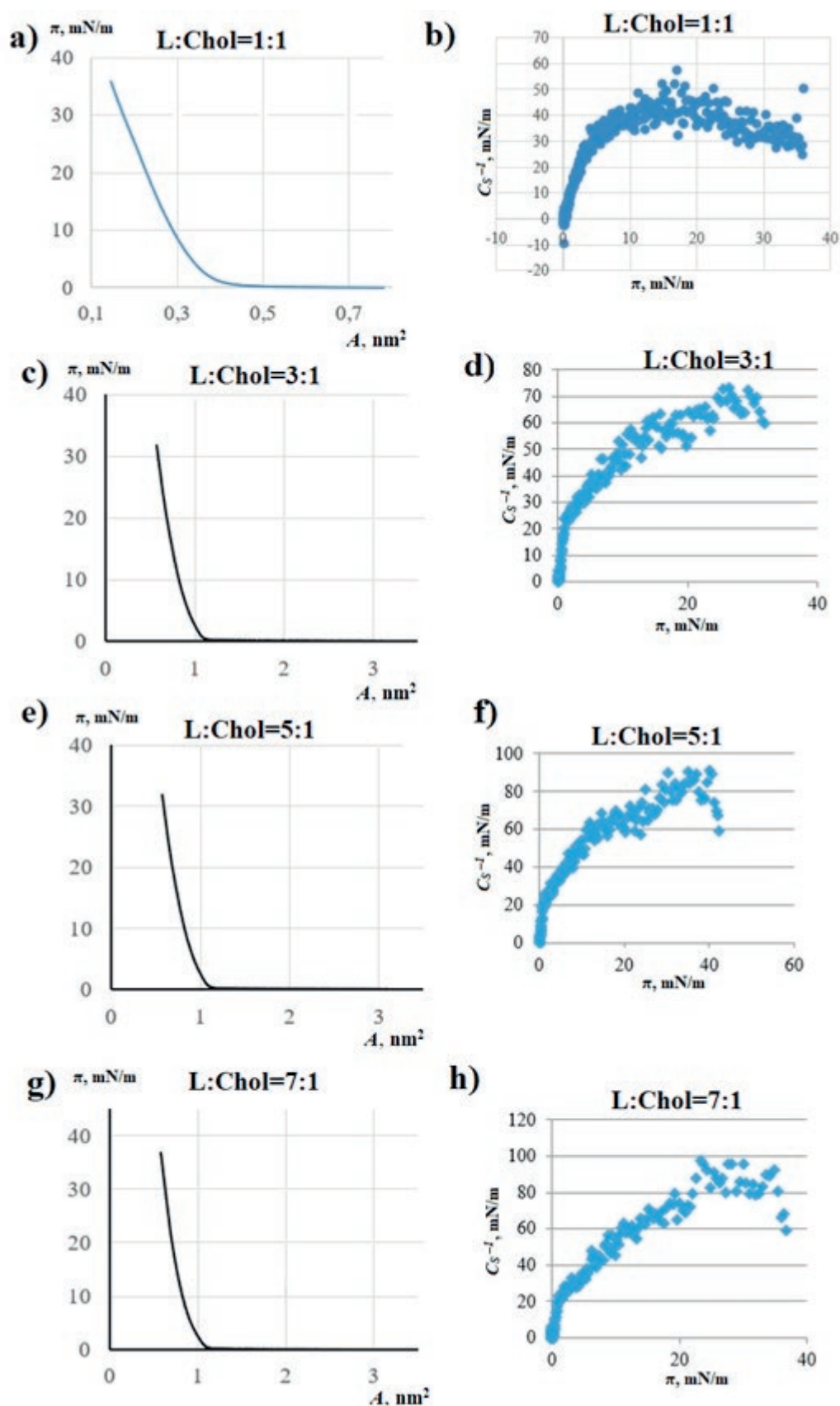


Fig. 2. Compression isotherms and compressibility moduli of mixed lipid monolayers with Lipoid S100 (L) – cholesterol (Chol) ratios of (1:1 – a, b; 3:1 – c, d; 5:1 – e, f; 7:1 – g, h)

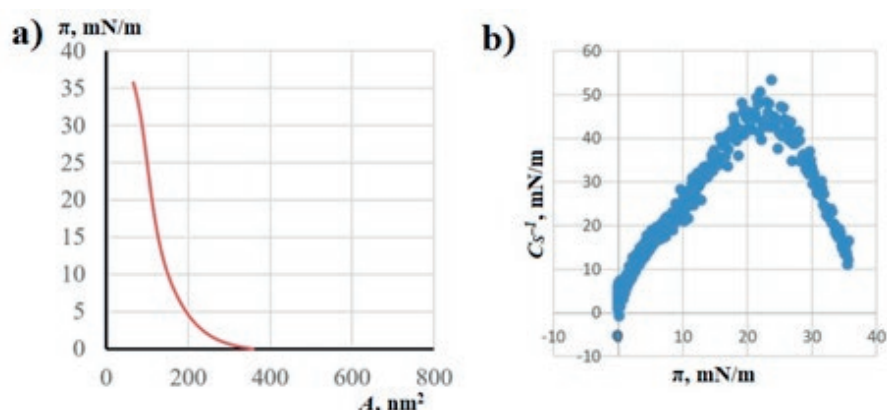


Fig. 3. Compression isotherm and compressibility modulus of monolayers of the MMA-BMA-DMAEM copolymer

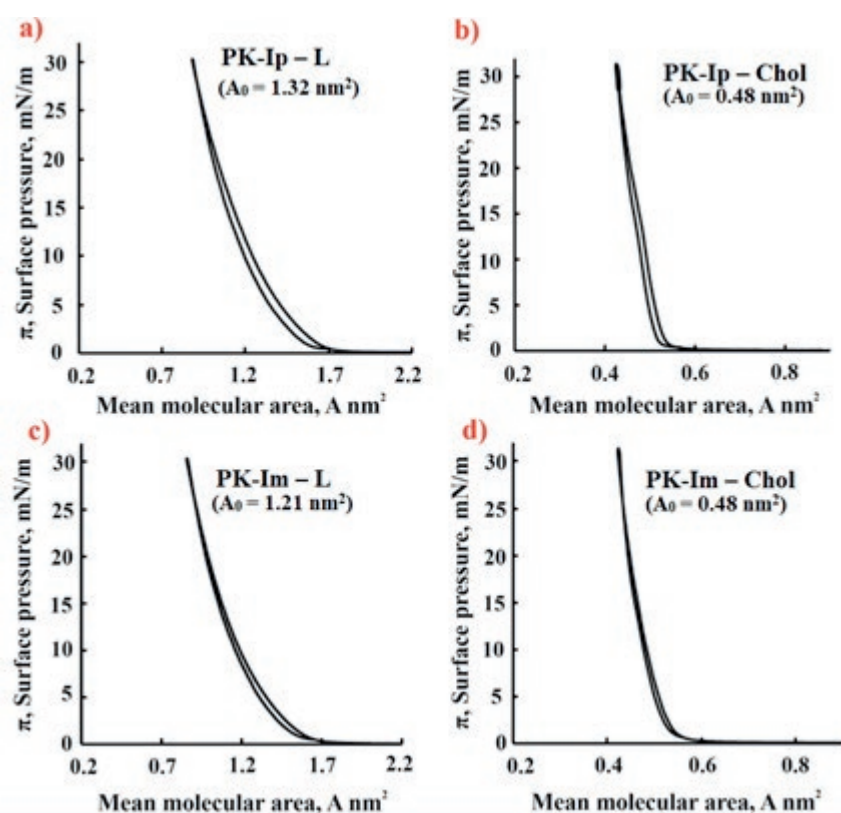


Fig. 4. Hysteresis curves of mixed monolayers of PK-Ip ionene polymer with Lipoid S100 (a), PK-Ip ionene polymer with cholesterol (b), PK-Im ionene macromonomer with Lipoid S100 (c), and PK-Im ionene macromonomer with cholesterol (d)

monolayers. The minimum hysteresis of the compression-tension curves of mixed monolayers was as follows: PK-Ip – Lipoid S100 ($\Delta A_0 = 0.07 \text{ nm}^2$); PK-Im – Lipoid S100 ($\Delta A_0 = 0.01 \text{ nm}^2$); PK-Ip – cholesterol ($\Delta A_0 = 0.01 \text{ nm}^2$); and PK-Im – cholesterol ($\Delta A_0 = 0.01 \text{ nm}^2$). These data indicate stable and similar states of all examined monolayers, and that polymer and monomer monolayers had the same structure. Table 1 summarizes the characteristics of mixed

“copolymer (MMA-BMA-DMAEM) – lipid” Langmuir monolayers. The data indicate that stable mixed monolayers form when the Lipoid S100 : cholesterol ratio did not exceed 3:1. Further increasing the Lipoid S100 proportion beyond this point did not enhance monolayer stability.

3.3. Colloidal-chemical properties of the studied lipoplexes

One of the problems of the formation of hybrid polymer-lipid nanoparticles (or lipoplexes)

Table 1. Properties of mixed monolayers of copolymer on lecithin and on a mixture of lipids (calculated by lipids)

Monolayer	L:Chol	$V_{\text{polymer}}, \mu\text{l}$	A_0, nm^2	Compressibility	
				$C_s^{-1}, \text{mN/m}$	$\pi, \text{mN/m}$
L	1:0	–	1.40 (L)	56	28
L–P	1:0	5	1.90 (L)	61	23
L–P	1:0	10	2.30 (L)	60 32	19 39
Chol	0:1	–	0.48 (Chol)	228	31
Chol–P	0:1	5	0.84 (Chol)	71 46	22 41
Chol–P	0:1	10	1.04 (Chol)	55 40	20 42
Chol–L	1:1	–	0.34 (Chol)	45	16
Chol–L–P	1:1	5	0.57 (Chol)	50	21
Chol–L–P	1:1	10	0.65 (Chol)	51 24	15 39
L–Chol	3:1	–	0.95 (L)	70	30
L–Chol–P	3:1	5	1.68 (L)	55	25
L–Chol–P	3:1	10	2.43 (L)	58	18
L–Chol	5:1	–	0.95 (L)	67 87	17 36
L–Chol–P	5:1	5	1.54 (L)	65	26
L–Chol–P	5:1	10	2.54 (L)	60 22	17 37
L–Chol	7:1	–	0.85 (L)	90	30
L–Chol–P	7:1	5	1.90 (L)	57 28	23 43
L–Chol–P	7:1	10	2.50 (L)	58 22	17 37

* L – Lipoid S100, Chol – cholesterol, P – copolymer (MMA-BMA-DMAEM)

is their tendency to aggregate resulting in instability. Accordingly, determining the size of nanoparticles, particularly their hydrodynamic radius can be used to monitor the stability of nanoparticles. Additionally, the size of lipoplex particles (from 100 to 200 nm) affects their surface charges. The assessment of the zeta potential prior to the formation of stealth lipoplexes can also be used as a standard method of analysis. The inclusion of hydrophilic polymers (commonly modified polyethylene glycols) in the aqueous phase intended to ensure “stealth” properties during the second stage of lipoplex formation significantly changes the surface charge. As a

rule, without modified PEGs, hybrid polymer-lipid nanoparticles have a high positive or negative charge, which determines their stability. After PEGylation, the particles tend to acquire an insignificant charge. In this regard, when developing technologies for producing lipoplexes, the charge of the particles is controlled before and after PEGylation. Table 2 shows the zeta potential values of poly(MMA-BMA-DMAEM)-lipid nanoparticles in water. Negative zeta potential values characterize the stability of the system consisting of the main components of the future nanocontainer and the small size of the nanoparticles.

Table 2. Colloidal properties of the obtained nanoparticles poly(MMA-BMA-DMAEM)-Lipid

N	L, mg	Chol, mg	P *, mg	Zeta potential, mV	Size, nm
1	200	50	200	-36.95±1.02	51
2	16	4	200	-27.68±4.06	73
3	10	10	200	-44.93±2.41	38
4	200	50	200	-34.95±1.54	49
5	10	10	200	-39.02±1.43	41
6	26.5	13.5	200	-49.68±3.96	32
7	132.8	67.2	40	-39.81±2.42	41
8	186	94	40	-53.69±1.79	31
9**	132.8	67.2	40	-15.23±1.23	73

Notes: * P = poly(MMA-BMA-DMAEM) copolymer (Mw. 89000); L – Lipoid S100, ** Liposome formation medium – phosphate buffer solution, pH 7.4

The zeta potential value decreased when the lipid-polymer film was dispersed in a phosphate buffer solution (pH 7.4). Since the obtained polymer is pH-sensitive and dissolves at pH 5, it is expected to dissolve at endosomal acidic pH, so it is preferable to form the nanocontainer core at pH 8 or higher. The charge sign of the hybrid lipid-polymer particles also affects their stability and distribution in the body. For example, liposomes containing dioleoyloxytrimethylammonium propane, which carry a positive charge, enhance the accumulation of antitumor drugs in the abdominal cavity of test animals. This effect is achieved due to the delayed release of the active substance and the resistance of these nanoparticles to macrophages. We have shown that nanoparticles based on an ionene polymer make it possible to obtain nanoparticles with a high positive charge (Table 3), which is a good indicator for predicting the stability of these particles.

4. Conclusion

In this work, we have shown that a number of polymers, such as poly(MMA-BMA-DMAEM) copolymer and ionene polymers (PK-Im and PK-Ip), combined with lipids (Lipoid S100 and

cholesterol) form genuine mixed layers that are most stable at various surface pressures. This stability is particularly pronounced at Lipoid S100: cholesterol molar ratio of 3:1, indicating a stable surface film under these conditions. In addition to the molecular area and collapse pressure, the compressibility modulus (C_s^{-1}) can be used as a stability control parameter with a minimum value of 50 mN/m. Nanoparticles of the MMA-BMA-DMAEM-lipid composition exhibited a strong negative charge (from -15 to -45 mV) and had small sizes in ranging from 32 to 73 nm. An alternative composition of stable lipid-polymer nanoparticles was a combination of lipids with positively charged ionene polymers, forming nanoparticles with a high zeta potential (from +8 to +49 mV).

Thus, we have proposed a method for predicting the compatibility and miscibility of the main components in hybrid lipid-polymer nanoparticles and demonstrated it using the (MMA-BMA-DMAEM) copolymer, ionene polymer (PK-Ip), Lipoid S100 lecithin, and cholesterol. This approach employs Langmuir monolayer technique and a method for preparation of lipid-polymer nanoparticles.

Table 3. Properties of nanoparticles with ionene polymer PK-Ip*

N	Lipoid, mg	Chol, mg	Ionene, mg	L:Chol	Lipids:ionene	Zeta potential, mV
1	8	32	40	1:4	1:1	+48.89±0.91
2	3	32	10	1:4	4:1	+26.25±2.92
3	20	20	10	1:1	4:1	+24.55±1.16
4	10	10	200	1:1	1:10	+8.02±1.55

Notes: * Lipids are dissolved in 10 ml of chloroform; ionene – in 10 ml of water

Contribution of the authors

The authors contributed equally to this article.

Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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