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LIPOSOME STABILITY DEPENDENCE ON FATTY ACID LECITHIN COMPOSITION AND SUNFLOWER PHOSPHOLIPIDS

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Phospholipids play an important physiological role being components of biologically active membranes of animals and humans nerve cells. The conditions of separation of sunflower phospholipids have been defined by the method of high performance liquid chromatography. Procedurer for quantitative determination of phosphatidylcholine, phosphatidylethanolamine and phosphatidyl inositol has been developed. It was done under normal phase mode and according to external standards and the order of exit. Phospholipid composition of sunflower lecithin has been identified and phospholipid fatty acid composition has been determined by tandem mass spectrometry. The presence of antioxidant α-tocopherol has been determined in liposomes composition, stipulating for maintaining stability of liposomal substance. Membrane-protecting effect of α-tocopherol is associated with its participation in arrangement of the membrane structure through direct interaction of its side isodental chain with polyunsaturated fatty acids of phospholipids, which leads to denser packaging of mitochondrial membranes and rising of increased resistance to lipid peroxygenation process effect.

Key words: lecithin, sunflower phospholipids, fatty acids, liposomes.

The term "lecithin" was used for lipids, received from egg yolk. Later, this term was applied as a synonym for specific phospholipid — phosphatidylcholine, which is the main component of the fraction of egg yolk and soybeans phosphatides. Having regards to some medical issues, the term "lecithin" was referred to -1,2-diacylglycero-3-phosphatidylcholine (L-α-lecithin). In contrast to this definition, in the industry and trade (particularly in the technology of drugs and food additives) the term «lecithin» is used to determine the mix of neutral lipids (triglycerides, a small amount of "free" fatty acids and sterols), polar lipids (phospho — and glycolipids) and carbohydrates.

At present, the most optimal is industrial use of liposomal preparations of natural phospholipids (soybeans, flax, canola, corn, sunflower, hazelnut, egg yolks, milk, salmon), since the introduction of liposomes are based on natural raw lecithin or phosphatidylcholine — ether phosphoric acids and fatty acids — is not associated with the risk of toxicity, immunogenicity and allergic reactions.

The stability of the liposomes can conveniently be divided into physical, chemical and biological stability being interrelated. As a rule, the term of liposomes stability is determined by their physical and chemical stability (uniformity of size distribution, encapsulation efficiency and minimal degradation of all components respectively). Preparations in liposomal form can become stable through optimizing their size distribution, pH and ionic strength, as well as due to addition of antioxidants and chelating agents [1].

Considering the fact that phospholipids usually form bilayer basis their chemical stability is deemed very important [2–4]. Two types of chemical reactions can affect the stability of the phospholipid bilayer: hydrolysis of esters that connect fatty acids with glycerol and unsaturated lipid acyl chains peroxygenation (if any).

The oxidation and hydrolysis of lipids can lead to liposomal preparations quality

degrading [4, 5]. In addition, physical processes like aggregation/flocculation affect the shelf life of liposomes, and may lead to loss of biologically active targeting substances and changes in the liposomes size [6]. The biological stability of liposomes depends on the presence of structures like proteins which interact with liposomes, and the route of liposomes entry. Permeability and stability of the liposomes are dependent on the lipid bilayer rigidity. The choice of lipids, in turn, depends on the temperature of the phase transition, which depends on the length of acylic chain. Gel-fluid phase transition occurs in a narrow temperature range for pure lipid (so-called phase transition temperature T_C). T_C depends on the length of the side chains and the degree of unsaturation of fatty acids as well as the polarity of the main groups. Lipids with long acyl chains are often used to generate liposomes, as they have a high phase transition temperature. However, the stability of liposomes cannot be achieved using only one type of lipids. The stability of liposomes can be achieved through a combination of lipids or inclusion of other substances [1]. Liposomal preparations of phospholipid nature start to be used widely in medical practice, so the vital issue of this new class of drugs deals with raw materials sources and methods of phospholipids obtaining. There are several groups of phospholipids that can be used in the preparation of liposomes, namely: phospholipids from natural raw materials, modified natural materials, semi-synthetic, synthetic, and those with natural "heads" [7–9]. Currently, there exist two approaches to solving the problems of availability of these compounds. The first approach is bio-inspired or chemical (enzymatic) synthesis (and combinations of these methods). The second is the selection of phospholipid fractions and their mixtures containing natural raw materials using bio-inspired methods.

At present, the use of natural phospholipids in production of liposomal preparations (from soybeans, flax, rapeseed, corn, sunflower, hazelnut, yolks of eggs, the milt of salmonid fishes) sounds the most optimal, since the introduction of liposomes based on natural raw materials involves no risk of toxicity, immunogenicity and allergic reactions [7, 9]. In addition, there are no restrictions on the use of natural lecithin in food products, cosmetics and medicines both in the European Union and in the instructions of Food and Drug Administration, medicines and cosmetics (FDA) of the USA [3, 10]. The macro and fatty acid composition of fat-free sunflower lecithin and sunflower lecithin impact on stability of liposomes have been researched in this scientific paper.

Materials and Methods

The objects being researched. Dry sunflower fat-free lecithin — a mixture of phospholipids, phosphatidylcholine (PC), extracted with ethanol or isopropanol — all belong to "PJSC DOIREA" Ukraine.

Instruments and equipment. High performance liquid chromatography (HPLC) "Dionex Ultimate 3000", Dionex. The following items enter into the chromatograph composition: pump LPG-3400A; spectrophotometric detector VWD-3400; Column heating oven TCC-3000; Autosampler WPS; column Ultra ll Silica, 3 μm, 100×4.6 mm. Triple quadrupole mass spectrometer API 3200 LC/MS/MS System, AB Sciex. Water clean-up system Direct-Q, Millipore. Weigh-scales Radwag, XAS 220/C.

Reagents and solvents. Deionized water; chloroform, HPLC grade, Merck; Methanol, HPLC grade, Merck; orthophosphate acid, HPLC grade, Merck; acetonitrile, HPLC grade, Merck; hexane, HPLC grade, Merck; benzene, HPLC grade; standard version of phospholipids (Phospholipid Mixture for HPLC from Soybean, Supelco, P3817-1VL, varied conc. in chloroform, L-α-PC 1500 mkg/ml, L-α-PE 1200 mkg/ml, L-α-PI (As ammonium salt), 900 mkg/ml, L-α-LPC 300 mkg/ml); ethanol farm.; 300 mkg/ml); TLC Silica gel 60, Merck; Pro Analysi; Aluminium oxide for chromatography, basic. Brockmnn I, 50–200 μм. Acros Organics.

Preparative discharging of phosphatidylcholine. It was found in previous research that phospholipids are the best separated using basic aluminum oxide. When PC discharging through preparative chromatography using basic aluminum oxide is performed. In this case aluminum purity equals more than 90% and lysophosphatidylcholine (LPC) can be extra mixed. Column is used to separate the substances, the ratio of length to inner diameter is equal to 20. Dense layer of cotton wool 1 cm thick and paper filter of relevant diameter was placed at the bottom. The column is filled with a suspension of basic aluminium oxide in a mixture of chloroform: methanol (1:1) for 1–2 days prior to separation of phospholipids and filter paper was placed on top. The portion of 30 mg of phospholipids per 1 g of dry sorbent was taken to separate the mixture. Phospholipids were dissolved

in a mixture of methanol-chloroform (1:1) and then brought into the column. PC with admixture of LPC was washed with two full columns volumes. 75% of the first volume was rejected. As soon as PC was discharged, the column was washed with one volume of the same mixture. Phosphatidylethanolamine (PE) mixed with phosphatidyl serine (PS) was washed with 2–3 volumes of ethanol-chloroform-water (5:2:2). The first volume was rejected. Both the effluents were evaporated to dryness with a rotary evaporator. The discharged volume was 80–90% in relation to the theoretically possible.

High performance liquid chromatography (*HPLC*). Detection of phospholipids followed by HPLC separation is generally carried out by absorption in the ultraviolet region of the spectrum or refractometrically. Phospholipids can be identified chromatographically both in native form and in the form of derivatives. Majority of membrane, barrier and superficial lipids have absorption band in the ultraviolet region [11], but the molar extinction factor of saturated lipids and monoene lipids is relatively small. Only lipids with a high content of unsaturated bonds have a significant extinction factor. Unmodified lipids are commonly found in absorption band of the UV region in the range of 203–213 nm.

Since a large number of organic solvents are strongly absorbed in the 200–215 nm range, a mixture of acetonitrile/ methanol/ water or hexane/isopropanol/water is used most often. Usually selectivity is increased as a result of appropriate selection of the effluents and by adding thereto acids or other ion modifiers. The mixture of acetonitrile, methanol and concentrated phosphoric acid and water was used as effluent for chromatographic separation of phospholipids through the column Ultra ll Silica, 3 micron, 100 × 4.6 mm, which enabled to determine quantitative volume of main sunflower lecithin components. Based on the performed tests, it was found that the following composition of mobile phase was the best to separate the substances as A:B = 99.6:0.4, where A — CH_3CN:CH_3OH: H_3PO_4 = 490:5:5, B — H_2O. The effluent was prepared as follows: 490 ml of acetonitrile, 5 ml of methanol and 5 ml of concentrated orthophosphoric acid were carefully mixed in a separate flask destined for mobile phase. Conditioned water was poured into another flask. The components A and B were mixed using a pump based on previously set program (isocratic mode). Minor variations in temperature (20–25 °C) practically produced no effect on the obtained results. The optimum indices were the flow rate — 1 ml/min and the detection wavelength — 208 nm. The sample was pre-filtered through polytetrafluoroethylene (PTFE) filter which resembled a spray nozzle with a diameter of pores of 0.22 microns. After each analysis made, the column was washed for 10–15 min at a flow rate of 1 ml/min.

Tandem mass spectrometry. Identification of phospholipids in sunflower lecithin was performed using tandem mass spectrometry method. Ionization method presented electrochemical spraying in the positive (identification of phosphatidylcholine) and in the negative (other phospholipids) mode (ESI^+, ESI^-). The identification of phospholipids groups was performed based on the availability of molecular ions of substances. Fatty acid composition and the presence of specific structural fragments (residues of inositol, glycerol, etc.) were confirmed based on the presence of characteristic product ion fractions-spectrum fragmentation of parent ions (in MS2 spectra). The group of phosphatidylcholines was identified in positive ionization mode through scanning product ion precursor according to ion ratio mass/charge 184 (the remainder of phosphatidylcholine). The Solution of sunflower lecithin with concentration of 0.05 mg/ml in chloroform system — a solution of formic acid 10 mmol/l in methanol = 1: 1 were injected with infusion method. Ionizing voltage equaled 4500 V, the source temperature — 300 °C, DP 50, CE 30. Full scanning spectra were in the mass range from 50 to 1000 a.o.m.

The formation of liposomes. Liposomes were prepared using an ultrasonic homogenizer with sonication method (Techpan UD-20) at a frequency of 22 kHz and ± 0.165 extruded using a hand extruder LiposoFast-Basic, AVESTIN. Preparing solutions of phospholipids followed the methods of [7].

The presence of α-tocopherol was determined in accordance with GOST 30417-96. Vegetable oils. Methods for determination of vitamins A and E mass fractions.

Statistical analysis of the results was made by calculating averages the average rates, their standard deviations and errors using Student's test. The differences were considered statistically-valid at $P < 0.05$.

Results and Discussions

"Raw" vegetable oils in addition to spare lipids (acetylglycine) include other groups of lipids (phospholipids, waxes) as well as products of hydrolysis and oxidation of lipids and the substances that determine the color, smell and taste of oil. Unwanted components of oil raw material are removed when refining. Oil refining begins with water treatment — hydration (extraction of hydrophilic compounds, primarily phospholipids). Oil subjected to heating and vigorous stirring is mixed with the required amount of water. The mixture is aged to ensure coagulation of phospholipids (phosphatides) and phosphatide emulsion is separated from hydrated oil. Phosphatides (phosphatide concentrate or liquid lecithin) being dried under vacuum to a moisture content of 90% are considered commercial products. Fat-free lecithin is obtained from phosphatide concentrate by extraction with acetone, the lecithin of the kind is then dried and grinded. Table 1 shows the main characteristics of fat-free lecithin obtained according to the procedure [7] from sunflower phosphatide concentrate by extraction with acetone. As shown in the Table. 1 dry fat-free lecithin phosphatide is actually isolate (containing 98% phosphatides). Phospholipid composition of obtained fat-free sunflower lecithin (Fig. 1) was defined using HPLC method. The chromatogram shows that the peaks of the main components are symmetrical.

Regarding the substances whose content is relatively low, the peaks have slightly extended rear slope, which may indicate these compounds high affinity to silica gel. They could be lysoforms of phospholipids. It is clearly seen that several substances whose retention time is less than phosphatidyl inositol (PI) retention time, were not divided. It was established previously by TLC [12] that the sunflower lecithin contains a significant amount (at PC) phosphatidic acid (PA). Using thin-layer chromatography [12], it has been earlier discovered that sunflower lecithin contained a significant ammount of phosphatidic acid (at PC level). However, after the high intensity peaks PI, other peaks are not observed, except identified ones. Probably, PA emerges earlier as compared with PI.

The time span of components retention is slightly larger than that one of the standard substances, which can be attributed to different fatty acid composition of soybean phospholipids (the standard is made from soy phospholipids) and sunflower phospholipids.

Owing to the fact that mainly phosphatidylcholine solutions are used to generate liposomes, extraction was conducted using ethyl and isopropyl alcohol. Chromatogram of 50% PC solution in ethanol given in Fig.2. It shows that PC content in the

Table 1. **Key features of fat-free sunflower lecithin**

Index name	Description and rate	Results
External view	Loose powder mass without impurities	Relevant
The smell and taste	Tasteless and odorless. Faint specific taste and smell of sunflower oil is allowed	Relevant
Color	From yellow-gray to fawn colored	Relevant
Moisture content,%, not exceeding	1.4	0.4
Oil content, %, not exceeding	1.5	1.0
Peroxide value mmol (½O_2)/kg, not exceeding	10.0	1.5
Acid value, mg KOH/g, not exceeding	32.0	15.0
Mass concentration of substances insoluble in toluene, %, not exceeding	2.0	0.6
Mass concentration of phospholipids, %, not exceeding	95.0	98.0
pH of 1% aquatic solution	6 — 7	6.4

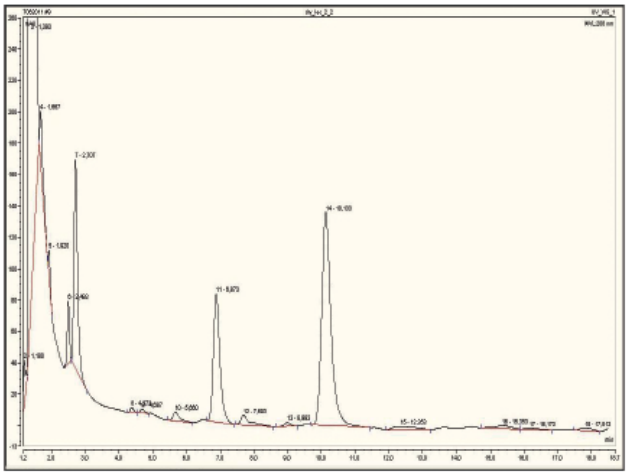

Fig. 1. **Chromatogram of sunflower lecithin**
Conditions of chromatography: v = 1 ml / min, t = 20 °C, λ = 208 nm column: Ultra ll Silica, 3 micron, 100×4.6 mm mobile phase: isocratic mode, A:B = 99:1; *A* — CH_3CN:CH_3OH:H_3RO_4 = 490:5:5 (vol.%) B — H_2O. Retention time span: 2,707min — PIX; 6.873 min — PE; 1 min 0.133 — PC.

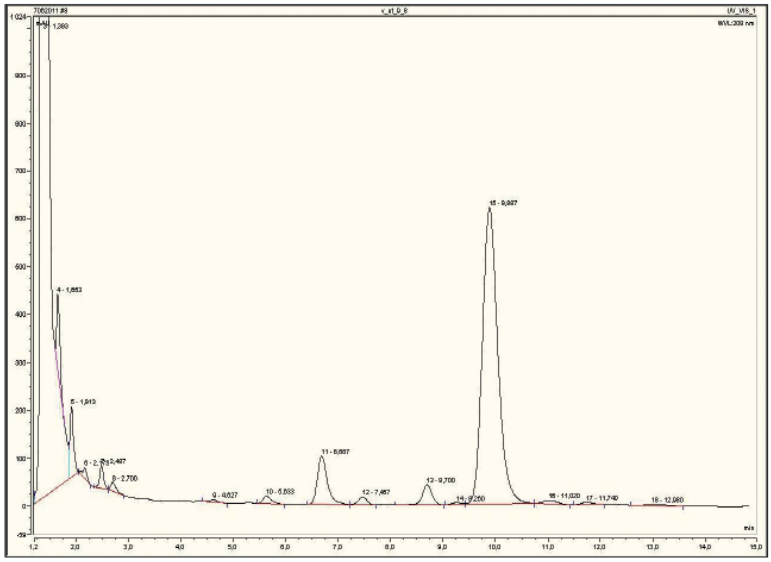

Fig. 2. **Chromatogram for PC (50% solution) extracted from sunflower lecithin by ethanol alcohol**
Conditions of chromatography: v = 1 ml / min, t = 20 °C, λ = 208 nm column: Ultra ll Silica, 3 micron, 100×4.6 mm mobile phase: isocratic mode, A:B = 99: 1; A — CH_3CN:CH_3OH:H_3PO_4 = 490: 5: 5 (vol.%), B — H_2O. Retention time spans: 2.460 min — PI; 5.973 min — PE; 8.753 min — PC

extract is big enough, but it is poorly cleared. A certain number of PE is available. The time span of components retention is a little more as compared with the standard, but slightly less than that one for the components of fat free lecithin. This can be explained by the fact that the sample resembled a 50% solution in ethanol and its trace amounts play some part in the division.

Chromatogram of 50% PC solution in isopropyl alcohol shown in Fig. 3 indicates that the PC content in the extract is large, but admixtures are still present. The main admixture is PE. However, as compared to the extract in ethanol, other contaminants are fewer in number. Retention time spans do not quite match the standard mixture retention time spans, they are somewhat smaller. This is explained by isopropanol impact.

Preparative-scale chromatography was used to obtain pure PC isolated from fat-free lecithin using a column with aluminum oxide. The chromatogram for PC, obtained with this method is given in Fig. 4.

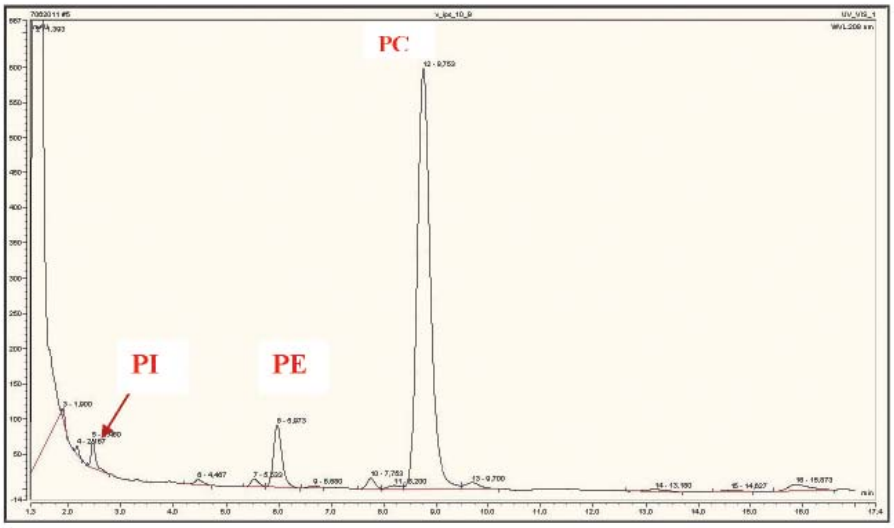

Fig. 3. **Chromatogram for PC (50% solution) extracted from sunflower lecithin by isopropyl alcohol**
Conditions of chromatography: v = 1 ml / min, t = 20 °C, λ = 208 nm column: Ultra ll Silica, 3 micron, 100×4.6 mm mobile phase: isocratic mode, A:B = 99:1; A — CH_3CN:CH_3OH:H_3RO_4 = 490:5:5 (vol.%), B — H_2O. Retention time spans: 2.460 min — PI; 5.973 min — PE; 8.753 min — PC

Table 2. **The content of phospholipids in fat-free sunflower lecithin and cleared PC**

Sample	Phospholipid	Content of masses%
Dry fat-free lecithin	PC	29.4 ± 0.3
	PE	12.5 ± 0.4
	PI	19.2 ± 0.5
The extract of PC in ethanol	PC	63.7 ± 0.1
	PE	6.7 ± 0.2
	PI	1.4 ± 0.2
The extract of PC in isopropylalcohol	PC	48.5 ± 0.3
	PE	4.4 ± 0.1
	PI	2.2 ± 0.1
PC, cleared by preparative chromatography	PC	97.8 ± 0.8

Table 2 shows the quantitative amount of phospholipids in a dry fat-free lecithin, 50% extracts with isopropanol and ethanol, as well as in PC, cleared by preparative chromatography.

Identification of phospholipids in sunflower lecithin and phospholipids fatty acid composition was performed with tandem mass spectrometry. Electrochemical spraying in the positive (identification of phosphatidylcholine) and negative modes (identification of other phospholipids) was used. The analysis results of phospholipids composition determining with tandem mass spectrometry used under negative ionization mode is shown in Fig. 5. PI, PE and PA, and phosphoglycerol (PG) and their respective lysoforms were identified by the given spectrum by molecular ion masses.

The range of ions in positive ionization is shown in Fig. 6. PC, which is invisible in negative ionization, is clearly seen in this case. The weight of its main molecular ions was comprised 782.7 and 784.7. The molecular weight of choline balance was 520.4, confirming that they are the forms of phosphatidylcholine. The analysis results to deal with sunflower lecithin are provided in Table 3. PC, PE, PG, PA, RI and related lysoforms are included in the sunflower lecithin composition. The most common saturated fatty acid inherent in animals,

Table 3. **Identifying the components of sunflower lecithin**

Phospholipid	Molar ion	Remains of fatty acids
PI	833	16:0, 18:2
	857	18:2, 18:2
	861	18:0, 18:2
LPI	595	18:2
	571	16:0
PA	695	18:2,18:2
	671	16:0, 18:2
	699	18:0, 18:2
PE	738	16:0, 18:2
PG	769	16:0, 18:2
	721	16:0, 16:0
	745	16:0, 18:2
LPG	483	16:0
PC	782	18:2,18:2
	758	16:0, 18:2
LPC	520	18:2
	496	16:0

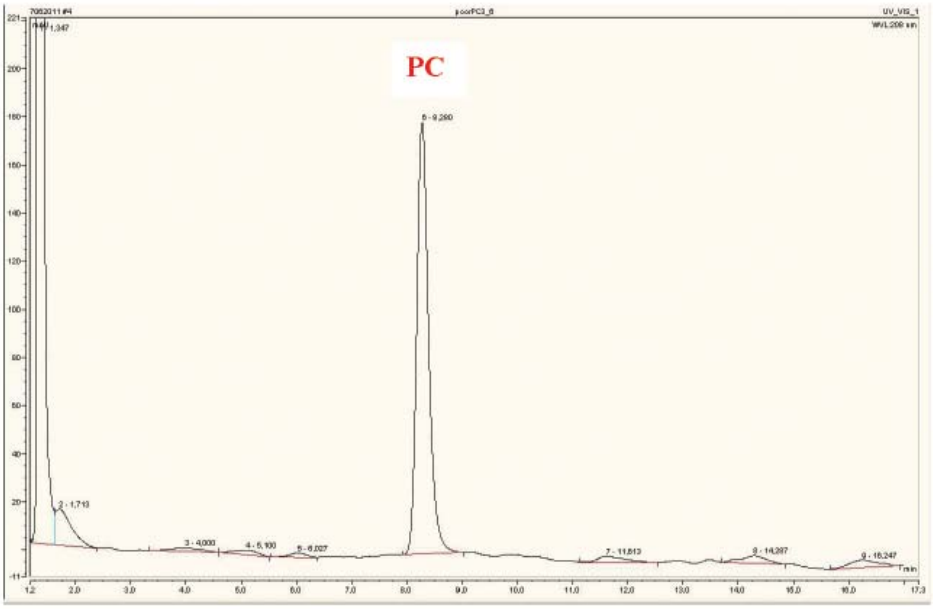

Fig. 4. **Chromatogram for PC extracted from sunflower lecithin by column chromatography based on main aluminum oxide**

Conditions of chromatography: v = 1 ml / min, t = 20 °C, λ = 208 nm column: Ultra ll Silica, 3 micron, 100×4.6 mm mobile phase: isocratic mode, A:B = 99:1; A — CH_3CN:CH_3OH:H_3RO_4 = 490:5:5 (vol.%), B — H_2O. PC Retention time span — 9.480 min

plants and microorganisms is palmitic acid (16:0). Stearic acid (18:0) is one of the essential fatty acids inherent in animals and some fungi and is a minor component of most plants. Oleic acid (18:1 omega-9) is the most common of monoene fatty acids in plants and animals. Palmitooleic acid (16:1 omega-7) is also widely occurred in animals, plants and microorganisms and is a major component of some vegetable oils. Linoleic acid (18:2

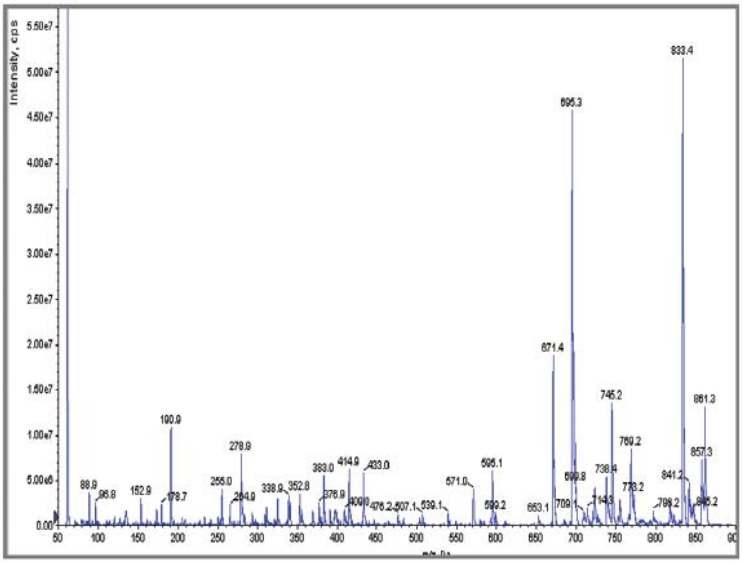

Fig. 5. **Sunflower lecithin ions spectrum ranging from 50 to 1000 a.m.u/ charge. Negative ionization**

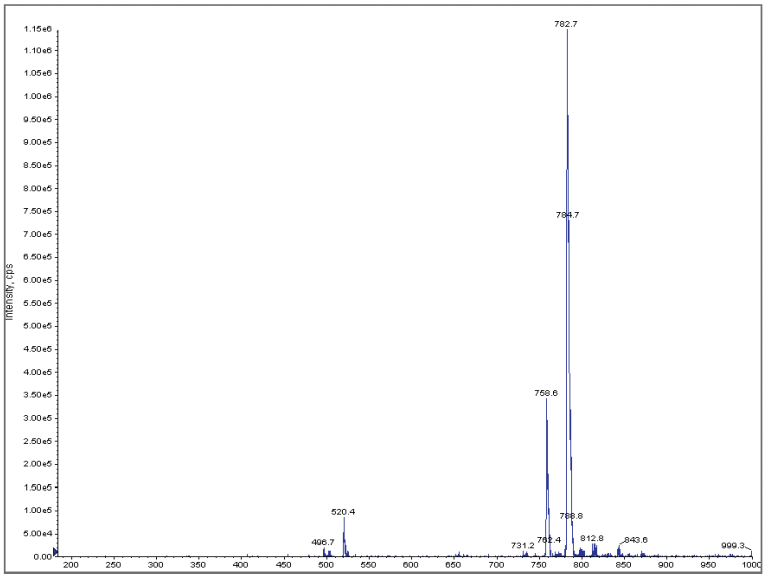

Fig. 6. **Sunflower lecithin ions spectrum ranging from 50 to 1000 a.m.u/ charge. Positive ionization.**

omega-6) is one of the essential fatty acids in plant lipids [13]. Palmitic and linoleic fatty acids are differently recombined in molecular composition of sunflower phospholipids. One of the most promising methods to deal with biotechnology implementation of phospholipids properties is creating the nanometer range structures — liposomes, synthetic analogs of biological membranes and their loading with drugs. Multiple functionalities of liposomes enable their successful inclusion in the scheme of medical treatment in the clinic [14]. The research of stability of liposomes (lipid peroxidation) obtained from sunflower lecithin was performed in the paper [7] as compared with liposomes, obtained from soybeans and egg yolks lecithin. The research results showed that liposomes obtained from sunflower lecithin proved to be the most stable.

The assumption was made in a number of studies that the stability of the liposomes could be linked to the phospholipid composition and different content pieces of unsaturated fatty acids, including oleic acid, which determine susceptibility to oxidation [8, 9]. However, the inspection results concerning the amount of fatty acids (in the form of ethyl ester derivatives)

using gas chromatography, showed that the content of unsaturated fatty acids of sunflower and soybeans lecithin, which were used in the experiments, were virtually the same (80% and 79%, respectively). In case of egg yolk lecithin, the total amount of unsaturated fatty acids reached 50%, with regard to the amount of oleic acid being about 5 times higher as compared to other lecithins. This is in compliance with the results concerning the properties of sunflower and soybeans lecithin [8] and egg yolk lecithin [9]. The presence of antioxidants contained in the extracts using 1,1-diphenyl-2-picrylhydrazil (DPPH Test) was also studied [15]. With regard to DPPH test system, the activity of free radicals was significantly less for sunflower lecithin than for soybean and egg yolk lecithin. Based on these results, the assumption was made that resistance of the liposomes obtained from sunflower lecithin to oxidative stress can be attributed to the effects of natural antioxidants. Research was conducted to identify α-tocopherol in sunflower lecithin samples according to GOST 30417-96. The content of α-tocopherol was discovered at 0.15% level, which is essential for maintaining stability of lyposomal substance.

The ability of α-tocopherol to dissolve only in fats and organic solvents hinders its entry to a cell. The decisive factor of α-tocopherol delivery into the cell through its membrane has to do with its inclusion in the bilayer of liposomes. Lipid bilayer typically contains PC, PE and α-tocopherol in a ratio of about 60:40:1. Phospholipids are favorable substrate for oxidation [17, 18], while they participate in a synergistic increasing in efficiency of tocopherol. Thus, prevention of membranes damage and decontamination of the process of free radical oxidation could be achieved through composition of phospholipids and polyphenols (α-tocopherol). The membrane-protective effect of α-tocopherol is associated with its participation in the membrane structure arrangement through direct interaction of its isodental side chain with polyunsaturated fatty acids as part of phospholipids. This interaction leads to denser packaging of mitochondrial membranes. As a result, their high resistance to lipid peroxygenation processes emerges due to the increased concentration of malondialdehyde and reduced activity level of antioxidant protection enzyme unit (superoxide dismutase and catalase).

REFERENCES

1. *Sulkowski W. W., Pentak D., Nowak K., Sulkowska A.* The influence of temperature, cholesterol content and pH on liposome stability. *J. Mol. Struct*. 2005, V. 744, P. 737–747.
2. *Yadav A. V., Murthy M. S., Shete A. S., Sfurti S.* Stability Aspects of Liposomes. *Ind. J. Pharm. Edu. Res.* 2011, 45 (4), 402–413.
3. *Liposome Drug Products.* Chemistry, Manufacturing, and Controls; Human Pharmacokinetics and Bioavailability; and Labeling Documentation. *Guidance for Industry*. U. S. Department of Health and Human Services, FDA, CDER. October 2015. Pharmaceutical Quality/CMC Revision 1.
4. *Rasti B., Jinap S., Mozafari M. R., Yazid A. M.* Comparative study of the oxidative and physical stability of liposomal and nanoliposomal polyunsaturated fatty acids prepared with conventional and Mozafari methods. *Food. Chem.* 2012, V. 135, P. 2761–2770.
5. *Araseki M., Yamamoto K., Miyashita K.* Oxidative Stability of Polyunsaturated Fatty Acid in Phosphatidylcholine Liposomes. *Biosci. Biotechnol. Biochem*. 2002, 66 (12), 2573–2577.
6. *Chen Irene A., Szostak Jack W*. Membrane growth can generate a transmembrane pH gradient in fatty acid vesicles. *PNAS*. 2004, 101 (21), 7965–7970.
7. *Shulga S. M., Glukh A. I., Glukh I. S.* Development of technology for obtaining dry lecithin from sunflower phosphatide concentrate. *Sci Innov.* 2012, V. 8, P. 62–71. (In Russian).
8. *Hollo J., Peredi J., Ruzics A. et al.* Sunflower lecithin and possibilities for utilization. *JAOCS*. 1993, V. 70, P. 997–1001.
9. *Budai L., Kaszas N., Grof P.* Liposomes for topical use: a physico-chemical comparison of vesicles prepared from egg or soy lecithin. *Sci Pharm*. 2013, V. 81, P. 1151–1166.
10. *WHO/CDS/NTD/IDM/*2007.4. Report of a WHO informal consultation on liposomal amphotericin B in the treatment of visceral leishmaniasis, Rome, Italy, 16 April 2005:World Health Organization, 18 p.
11. *Henschen A. K-P. Hupe, Lottspeich F., Voelter W*. High Performance Liquid Chromatography in Biochemistry. Edited by A. Henschen K-P. Hupe. *Verlag Chemie, Weinheim*. 1986. P. xiii + 638.
12. *Shulga S. M., Glukh I. S.* Dry powder lecithin and liposomal nanoemulsions from sunflower. *Abstracts of posters 10th ILPS Phospholipid Congress*, Rotterdam, the Netherlands, 16–18 September 2011, P. 6.
13. *Rustan A. C., Drevon C. A.* Fatty Acids: Structures and Properties. *Encyclopedia of life sci.,* John Wiley & Sons. 2005, P. 1–7. doi: 10.1038/npg.els.0003894.
14. *Raynor A., Jantscheff P., Ross T., Schlesinger M., Wilde M., Haasis S., Dreckmann T., Bendas G., Massing U*. Saturated and mono-

unsaturated lysophosphatidylcholine metabolism in tumour cells: a potential therapeutic target for preventing metastases. *Lipids in Health and Disease*. 2015, 14 (69), 1–15. doi: 10.1186/s12944-015-0070-x.
15. *Melnyk Andrii K., Sukhoveev Olexandr V., Kononets Lyudmyla A., Khilchevsky Olexandr M., Shulga Serhiy M., Kukhar Valery P., and Vovk Andriy I.* An EPR spin probe study of liposomes from sunflower and soybean phospholipids. *J. Liposome Res.* 2015, Early Online: 1–7, Informa Healthcare USA, Inc. doi: 10.3109/08982104.2015.1039031.
16. *Roselena S. Schuh, Fernanda Bruxel, Helder F. Teixeira.* Physicochemical properties of lecithin-based nanoemulsions obtained by spontaneous emulsification or high-pressure homogenization. *Quim. Nova.* 2014, 37 (7), 1193–1198.
17. *Schnitzer E., Pinchuk I., Lichtenberg D.* Peroxidation of liposomal lipids. *Eur. Biophys. J.*, 2007, V. 36, P. 499–515.
18. *Reis A., Spickett C. M.* Chemistry of phospholipid oxidation. *Biochim. Biophys. Acta*. 2012, V. 1818, P. 2374–2387.

ЗАЛЕЖНІСТЬ СТАБІЛЬНОСТІ ЛІПОСОМ ВІД ЖИРНОКИСЛОТНОГО СКЛАДУ ЛЕЦИТИНУ І ФОСФОЛІПІДІВ СОНЯШНИКУ

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Метою роботи було визначення стабільності ліпосом залежно від жирнокислотного складу лецитину та есенціальних фосфоліпідів соняшнику. Методом високоефективної рідинної хроматографії визначено умови розділення фосфоліпідів соняшнику і розроблено методику кількісного виявлення фосфатидилхоліну, фосфатидилетаноламіну і фосфатидилінозитолу в нормальнофазному режимі за зовнішніми стандартами та порядком їх виходу. Ідентифіковано фосфоліпідний склад лецитину соняшнику та визначено жирнокислотний склад фосфоліпідів за допомогою методу тандемної мас-спектрометрії. Встановлено наявність у складі ліпосом антиоксиданту α-токоферолу, що є умовою збереження їхньої стабільності. Мембранопротекторний ефект α-токоферолу пов'язаний з його участю в організації структури мембран через пряму взаємодію його бічного ізодентального ланцюга з поліненасиченими жирними кислотами фосфоліпідів, що сприяє більш щільному пакуванню мембран мітохондрій і підвищеній стійкості до процесів пероксидного окиснення ліпідів.

Ключові слова: лецитин, фосфоліпіди соняшнику, жирні кислоти, ліпосоми.

ЗАВИСИМОСТЬ СТАБИЛЬНОСТИ ЛИПОСОМ ОТ ЖИРНОКИСЛОТНОГО СОСТАВА ЛЕЦИТИНА И ФОСФОЛИПИДОВ ПОДСОЛНЕЧНИКА

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Целью работы было определение стабильности липосом в зависимости от жирнокислотного состава лецитина и эссенциальных фосфолипидов подсолнечника. Методом высокоэффективной жидкостной хроматографии определены условия разделения фосфолипидов подсолнечника и разработана методика количественного выявления фосфатидилхолина, фосфатидилэтаноламина и фосфатидилинозитола в нормальнофазном режиме в соответствии с внешними стандартами и порядком их выхода. Идентифицирован фосфолипидный состав лецитина подсолнечника и определен жирнокислотный состав фосфолипидов с помощью метода тандемной масс-спектрометрии. Установлено наличие в составе липосом антиоксиданта α-токоферола, что является условием сохранения их стабильности. Мембранопротекторный эффект α-токоферола связан с его участием в организации структуры мембран за счет прямого взаимодействия его боковой изодентальной цепи с полиненасыщенными жирными кислотами фосфолипидов, что способствует более плотной упаковке мембран митохондрий и появлению повышенной устойчивости к действию процессов пероксидного окисления липидов.

Ключевые слова: лецитин, фосфолипиды подсолнечника, жирные кислоты, липосомы.