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INTERACTION OF EUROPIUM CHELATES WITH LIPID MONOLAYERS

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The ability of novel anticancer drug candidates, europium coordination complexes (EC), to penetrate the phospholipid monolayer composed of dimyristoylphosphatidylcholine (DMPC) was studied using Langmuir monolayer technique. EC were found to insert readily into the lipid monolayer with penetration extent being dependent on both drug structure and initial surface pressure of the lipid film. Evaluation of the limiting surface pressure revealed that all drugs are capable of inserting into the cellular membranes.
KEYWORDS: europium complexes; anticancer drugs; Langmuir monolayers; membrane penetration

ВЗАИМОДЕЙСТВИЕ ХЕЛАТОВ ЕВРОПИЯ С ЛИПИДНЫМИ МОНОСЛОЯМИ

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С помощью метода монослоев Ленгмюра была исследована способность новых противоопухолевых препаратов, координационных комплексов европия (ККЕ), встраиваться в липидный монослой, сформированный из димиристоилфосфатидилхолина (ДМФХ). Было обнаружено, что ККЕ проникают в липидный монослой, и степень проникновения зависит как от структуры препарата, так и от начального поверхностного давления липидной пленки. Оценка критического поверхностного давления монослоя показала, что исследуемые препараты могут встраиваться в клеточные мембраны.
КЛЮЧЕВЫЕ СЛОВА: комплексы европия; противоопухолевые препараты; монослои Ленгмюра; встраивание в мембрану

ВЗАЄМОДІЯ ХЕЛАТІВ ЄВРОПІЮ З ЛІПІДНИМИ МОНОШАРАМИ

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За допомогою методу моношарів Ленгмюра була досліджена здатність нових протипухлинних препаратів, координаційних комплексів європію (ККЄ), вбудовуватися у ліпідний моношар, сформований з дімірістоїлфосфатидилхоліну (ДМФХ). Знайдено, що ККЄ проникають у ліпідний моношар, а ступінь проникнення залежить як від структури препарату, так й від начального поверхневого тиску ліпідної плівки. Оцінка критичного поверхневого тиску моношару показала, що досліджувані препарати можуть вбудовуватися у клітинні мембрани.
КЛЮЧОВІ СЛОВА: комплекси європію; протипухлинні препарати; моношари Ленгмюра; вбудовування у мембрану

Interactions of pharmacological agents with cell membranes play a fundamental role in both drug activity and delivery. According to the canonical Meyer-Overton rule, membrane lipids represent the target for the pharmaceuticals, and drug potency linearly correlates with the solubility in lipid phase. The important prerequisite for attaining the necessary therapeutic effect of a drug is its entrance into cell through plasma membrane. Irrespective of the mechanism of its uptake and action, membrane permeability of pharmacological compound is crucial for its targeted delivery [1]. In view of this, thorough investigation of the molecular level interactions between the drug and lipids seems to be essential aspect of drug design. Due to extremely complex composition of cellular membranes and ambiguity of the obtained results, model membranes of defined composition represent the convenient systems for analyzing the drug-lipid interactions, especially those occurring at lipid-water interface. Among a huge variety of existing model membranes, Langmuir monolayers are prized for their homogeneity, stability and planar geometry [2,3]. In addition, a range of variables like chemical nature and packing degree of lipid molecules, temperature, pH, ionic strength, subphase

composition, etc. can be readily modulated [4]. Analysis of the surface pressure – area isotherms, kinetic curves and surface pressure changes may provide quantitative information on the drug membrane affinity and penetration ability, influence of pharmacological agents on stability and permeability of lipid monolayers, etc. [1,4-7].

In the present work Langmuir monolayer technique has been employed to explore the penetration behavior of the novel anticancer drug candidates – europium (III) tris-β-diketonates – into dimyristoylphosphatidylcholine (DMPC) lipid membranes. EC are an asymmetric Eu(III) coordination complex with acetyl acetone ligands and a 1,10 phenanthroline motif. The organic chromophores of this complex are responsible for absorbing the excitation light and transferring the energy to the lanthanide. Taking into account the high hydrophobicity of this compound and its relatively small size (approximately 11 Å) in comparison with the lipid bilayer thickness (46 Å) EC are expected to be efficiently incorporated into the lipid phase. Indeed, the ability of europium chelates to penetrate the lipid bilayer has been demonstrated in our previous works, in particular, using several fluorescent probes [8,9]. However, utilization of external fluorophores is indirect method for tracing membrane incorporation of a certain compound, which can interfere with other processes occurring in the system, for instance, specific interactions between the probe and lipid bilayer or drug molecule. Langmuir monolayer studies outlined here represent more accurate detection of EC membrane penetration, and thus may emerge more detailed picture of drug-lipid complexation.

The purpose of this work is to study the lipid-associating ability of the novel potential anticancer drugs using the Langmuir monolayer technique.

MATERIALS AND METHODS

Materials

1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) was from Avanti Polar Lipids (Alabaster, AL). Eu(III) coordination complexes defined here as V3 – V12 (Fig. 1) were synthesized at the Faculty of Pharmacy and Chemistry, University of Sofia, as described previously [10]. All other chemicals were of analytical grade and used without further purification.

Monolayer experiments

Monolayer measurements were conducted using tensiometer DeltaPi-4 (Kibron, Finland). A Teflon trough (Multiwell plate, Kibron Inc., Espoo, Finland) was filled with 1.3 ml of filtered 10 mM HEPES buffer (pH 7.4). The subphase was continuously stirred with a magnetic bar during the measurement. Monomolecular lipid film was formed by careful spreading of DMPC chloroform solution at air/buffer interface with a Hamilton syringe. Surface pressure (π) was monitored with a Wilhelmy plate attached to a computer-controlled Langmuir film balance. After stabilization of applied monolayer to the desired initial surface pressure π_0, the drugs were injected into the subphase. All measurements were performed at ambient temperature. The increase in surface pressure (Δπ) was monitored until the stable value was reached. Δπ was plotted *vs.* π_0 for each drug, yielding straight line with negative slope and *x*-axis intercept equal to limiting surface pressure.

Thermodynamics of drug penetration into lipid monolayer

The thermodynamical description of drug penetration into lipid monolayer is based on classical Gibbs adsorption isotherm. Surface pressure (Π) is defined as:

$$\Pi = \gamma_0 - \gamma \tag{1}$$

where γ_0 and γ are the liquid/vapor surface tension of pure solvent and with adsorbed surfactant, respectively. The classical surface excess of insoluble species M can be expressed as:

$$\Gamma_M = \frac{1}{A_M} \tag{2}$$

where A_M is the area per molecule of the insoluble surfactant in the monolayer.

The surface excess of the soluble surfactant, Γ_S, is approximately equal to its surface concentration, at least for species that are strongly surface active. The chemical potential and the activity of the soluble surfactant are related by $d\mu_s = RTd\ln a_s$. For very dilute solutions, activity a_s is approximately equal to the molar concentration c_s of the soluble component in the subphase solution. Therefore,

$$d\mu_s = RTd\ln c_s \tag{3}$$

For a two component system Gibbs adsorption equation takes the form:

$$d\gamma = -\Gamma_M d\mu_M - \Gamma_s d\mu_s \tag{4}$$

or taken into account Eq. (1):

$$d\Pi = \Gamma_M d\mu_M + \Gamma_s d\mu_s \tag{5}$$

Assuming the adsorbed amount of M is fixed

$$\left(\frac{\partial\Pi}{\partial\mu_s}\right)_{\Gamma_M} = \Gamma_s + \Gamma_M\left(\frac{\partial\mu_M}{\partial\mu_s}\right)_{\Gamma_M} \tag{6}$$

Rearranging this equation results in

$$\left(\frac{\partial \Pi}{\partial \mu_s}\right)_{\Gamma_M} = \Gamma_s \left[1 - \Gamma_M \left(\frac{\partial \mu_M}{\partial \Pi}\right)_{\Gamma_M}\right]^{-1} \tag{7}$$

Change in excess Gibbs free energy at constant temperature and pressure is

$$dG = -Ad\gamma + \mu_s dm_s + \mu_M dm_M \tag{8}$$

where A is surface area, m_s and m_M are excess moles of soluble and insoluble (monolayer forming) components, respectively. Maxwell relations can be written as:

$$\left(\frac{\partial \mu_M}{\partial \gamma}\right)_{m_M, m_s} = -\left(\frac{\partial A}{\partial m_M}\right)_{\gamma} \tag{9}$$

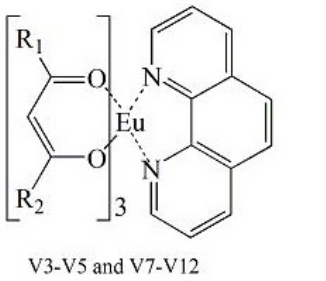
$$\left(\frac{\partial \mu_M}{\partial \Pi}\right)_{m_M, m_s} = \left(\frac{\partial A}{\partial m_M}\right)_{\gamma} = \bar{A}_M \tag{10}$$

where $\bar{A}_M$ is defined as partial molar area of the component M. Combination of the Eqs. (7), (9) and (10) yields:

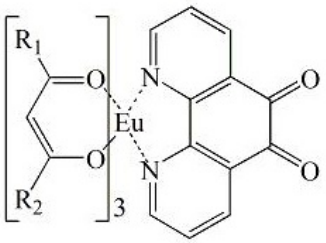
$$\Gamma_s = \frac{1}{RT}\left(\frac{A_M - \bar{A}_M}{A_M}\right)\left(\frac{\partial \Pi}{\partial \ln c_s}\right)_{A_M} \tag{11}$$

RESULTS AND DISCUSSION

During the past decades lanthanide complexes are placed in the focus of increasing research efforts because of their extremely attracting photophysical properties, such as exceptionally long lifetime, large Stokes' shifts, and line-like emission [11] arising from Laporte-forbidden *f-f* transitions in lanthanide ions [12]. These unique spectral characteristics created the necessary prerequisites for utilization of lanthanide chelates as luminescent materials [13], chemosensors [14], fluorescent labels [15], photoluminescence devises [16], etc. Moreover, lanthanides are successfully used in medicine as effective MRI contrast agents, hypophosphatemic agents for hemodialysis and palliative pharmaceuticals for osteosarcoma patients [17-20]. Recently medical applications of lanthanide complexes were expanded by introducing the newly synthesized europium compounds (EC) as novel anticancer drugs [10] (Fig. 1). Specifically, europium (III) tris-β-diketonates were shown to exhibit profound cytotoxic effect presumably arising from abundance of DNA-intercalating pharmacophore in their structure [11].



V3-V5 and V7-V12



V6

EC	R_1	R_2
V3	CH_3	CH_3
V4	CH_3, C_4H_3S	CH_3, CF_3
V5	C_6H_5	CH_3
V6	CF_3, C_4H_3S	C_4H_3S, CF_3
V7	C_6H_5	CF_3
V8	C_4H_3S, $C_{10}H_7$	CF_3
V9	C_7H_7O	CF_3
V10	C_6H_5	C_6H_5
V11	$C_{10}H_{12}NO$	CF_3
V12	C_6H_4Cl	CF_3

Fig. 1. Chemical structures of europium complexes

Since the main prerequisite for pharmacological action of the drug is its association with membrane lipids, in the present contribution we utilized lipid monolayer technique in attempt to get molecular-level understanding of EC therapeutical effect. The monolayer technique represents an extremely informative tool for mimicking the phenomena occurring at cellular membrane/extracellular medium interface.

Typically, compounds interacting only with monolayer surface induce minor changes in monolayer pressure. On the contrary, penetration into hydrophobic core of lipid monolayer induces profound alterations in π values. Fig. 2 shows the variation in surface pressure upon injection of EC into the subphase underneath DMPC lipid monolayer. As seen from this figure, addition of the drugs resulted in the marked increase of π value with time, indicating fast

monolayer penetration of these agents. Notably, kinetic profiles of EC penetration were found to depend on the drug structure. Specifically, for V4, V7, V8, V9 and V10, after initial increase and some fluctuations Δπ values reach the equilibrium, as can be judged from the plateaus attained around 11.2, 10.2, 10.8, 15.2 and 10 mN/m, respectively, indicating the termination of drug penetration. In contrast, in the case of V3, V5 – V7 and V12, Δπ slowly decreases after a steep increase up to the maximum values 14.5 mN/m for V3 and V6, 16 mN/m for V5 and 15.8 mN/m for V12. This effect can be explained by partial desorption of some EC molecules from the lipid monolayer [4]. Finally, in the case of V11 Δπ exhibited a saturation after reaching the maximum value and then showed continuous increase which may reflect the multilayer drug sorption.

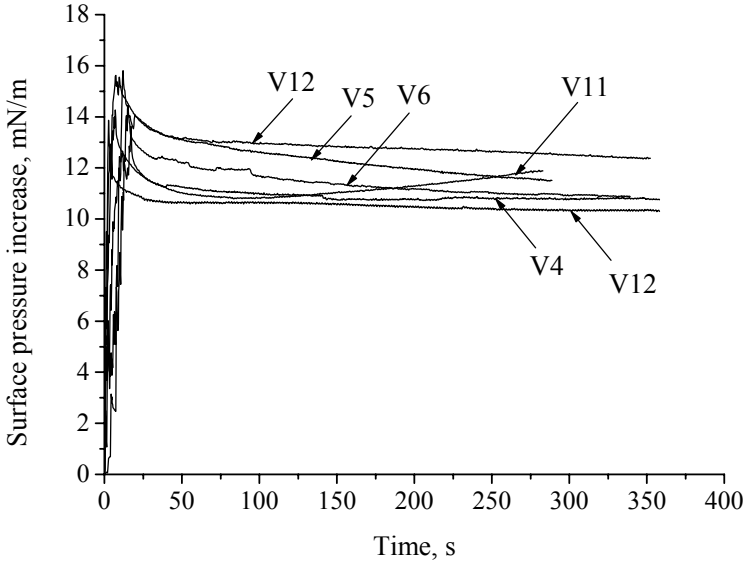


Fig. 2. Representative penetration kinetics of EC into DMPC monolayer

Of course, based on the results of monolayer experiments it is difficult to draw unambiguous conclusions on the origin of kinetic profiles observed here for EC. However, it cannot be excluded that the observed phenomena reflect modification of molecular architecture of lipid monolayer by europium complexes. Particularly, specific interactions of lanthanide molecular groups with lipid headgroups may modify hydrogen bonding network within the monolayer and destabilize it contributing positively (further increase in surface pressure, like for V11) or negatively (drug desorption from the monolayer, as was shown for V3, V5, V6 and V12) to the energy of drug-lipid complexation. However, to confirm the validity of the above assumptions, further studies are required. With the purpose to get closer to the details of drug desorption process we used linear regression model for analysis of post-penetration profiles for V3, V5, V6 and V12. The linear extrapolation of the penetration curves for these EC presented in Fig. 2 revealed that the time required for complete desorption (Δπ becomes zero) of the drugs from lipid monolayer is 445, 430, 470 and 475 s for V3, V5, V6 and V12, respectively. This time is considered as the time at which drug-lipid interactions are finished and monolayer re-attained its original surface pressure.

In order to obtain more detailed information about EC interactions with DMPC monolayers we examined the drug-induced changes in surface pressure as a function of π_0. The values of Δπ were found to decrease upon increasing initial surface pressure (Fig. 3).

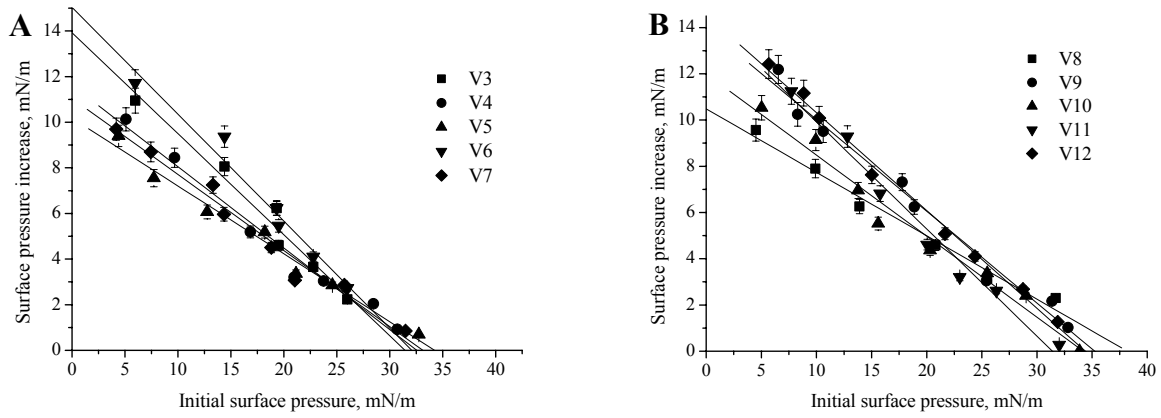


Fig. 3. Surface pressure increase as a function of initial surface pressure in the presence of EC

This finding is explained by tighter packing degree of lipid molecules in more compressed monolayers with higher π_0. This would prevent drug penetration into the lipid film, resulting in decreased $\Delta\pi$. Notably, at different initial surface pressures the drug rows showing the rise in $\Delta\pi$ were found to be different. For instance, at π_0=5 mN/m V10 displayed the lowest increase in surface pressure, while V5 showed the highest increase of this parameter. In turn, at π_0=30 mN/m the minimal and maximal rise in $\Delta\pi$ was found for V4 and V8, respectively. The observed phenomenon allowed us to suppose that there is no unique mechanism of EC – lipid association, and that complex interplay of the factors such as drug structure from one hand, and lipid monolayer physicochemical properties from the other hand, seem to play essential role in the drug membrane incorporation.

At the last stage of our study we quantitatively analyzed the plots of surface pressure increase *vs.* initial pressure presented in Fig. 3. Approximation of these curves by linear regression model yielded the value of limiting surface pressure (π_{lim}). Notably, different terms are currently used for π_{lim} [21]. Among them are exclusion pressure, critical insertion pressure, maximum insertion pressure, surface pressure cut-off, packing pressure, etc. In fact, all these terms reflect the boundary surface pressure at which the insertion of a certain compound into monolayer is energetically favorable, and beyond which no penetration occurs [4]. Table 1 summarizes the values of π_{lim} for different EC. It appeared that for all drugs limiting surface pressure is greater than 30 mN/m, the value corresponding to the surface pressure of biological membranes [3].

Table 1.

The values of limiting surface pressure for EC penetration into the lipid monolayer

EC	π_{lim}
V3	32.8±2.4
V4	33.1±2.5
V5	33.6±6.2
V6	32.7±3.1
V7	34.1±3.7
V8	39.3±5.2
V9	35.2±3.9
V10	33.9±3.4
V11	31.8±3.2
V12	34.8±2.4

This finding indicates that the examined compounds would readily penetrate into cell. The largest π_{lim} was revealed for V8, while the lowest – for V11, reflecting respectively the highest and the lowest affinity of these drugs for the membrane.

CONCLUSION

In conclusion, using the Langmuir monolayer technique we studied the association of the novel potential anticancer drug candidates, europium coordination complexes, with DMPC lipid monolayers. It was found that the examined drugs readily penetrate the lipid monolayer. Furthermore, based on the values of limiting surface pressure it was shown that all drugs would penetrate also cell membranes. However, V3, V5, V6 and V12 were shown to desorb from the lipid monolayer at approximately 8th minute of the interaction. Thus, there is a strong need for improvement of EC penetration into the monolayer without drug desorption that would result in the achievement of maximal therapeutic effect of these pharmacological agents.

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