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Functional nanoparticles for biomedical applications: a dsc study of membranotropic behavior

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Interaction of fullerene-containing silica nanoparticles (SiO_2–C_{60}, SiO_2–C_{60}–Pd) and DNA of natural origin (DNA and low molecular weight DNA — LmwDNA) with phospholipid model membranes was studied using differential scanning calorimetry (DSC). SiO_2–C_{60}, SiO_2–C_{60}–Pd and DNA had only minor effects on *L*-α-dipalmitoyl phosphatidyl choline (DPPC) membrane phase transitions, remaining essentially inert. LmwDNA induced noticeable changes in the DSC profiles, with the effects (increasing of the main phase transition temperature, significant peak broadening and splitting, vanishing of the pre-transition peak) increasing with concentration. No noticeable deviations from additivity could be noted under joint introduction of the nanosystems into DPPC membranes.

Keywords: nanoparticles, phospholipid membranes, differential scanning calorimetry, low molecular weight DNA, phase transition.

Функціональні наночастинки для біомедичних застосувань: дослідження мембранотропної поведінки методом ДСК. *О.М.Самойлов, Л.М.Лисецький, Н.О.Касян, М.Ю.Лосицький, О.А.Голуб, В.М.Ящук*

Методом диференціальної скануючої калориметрії (ДСК) досліджено взаємодію фулереновмісних наночастинок оксиду кремнію (SiO_2–C_{60}, SiO_2–C_{60}–Pd) та ДНК природного походження (DNA та низькомолекулярна ДНК — LmwDNA) з модельними фосфоліпідними мембранами. Наночастинки SiO_2–C_{60}, SiO_2–C_{60}–Pd та ДНК мали незначний влив на фазові переходи мембран *L*-α-дипальмітоїлфосфатидилхоліну (DPPC), виявляючи себе інертними стосовно DPPC мембран. LmwDNA викликала зміни у ДСК термограмах, причому ефекти (підвищення температури основного фазового переходу, значне розширення та розщеплення піку, зникнення піку передпереходу) збільшувалися з ростом концентрації. При спільному уведенні наносистем до DPPC мембран не було відмічено помітних відхилень від адитивності.

Методами дифференциальной сканирующей калориметрии (ДСК) исследовано взаимодействие фулеренсодержащих наночастиц оксида кремния (SiO_2–C_{60}, SiO_2–C_{60}–Pd) и ДНК природного происхождения (DNA и низкомолекулярная ДНК — LmwDNA) с модельными фосфолипидными мембранами. Наночастицы SiO_2–C_{60}, SiO_2–C_{60}–Pd и ДНК оказывали слабое влияние на фазовые переходы мембран *L*-α-дипальмитоилфосфатидилхолина (DPPC), будучи инертными по отношению к DPPC мембранам. LmwDNA вызвала изменения в ДСК термограммах, эффекты (повышение температуры основного фазового перехода, значительное расширение и расщепление пика, исчезновение пика предперехода) увеличивались с ростом концентрации. При совместном введении наносистем в DPPC мембраны не было отмечено заметных отклонений от аддитивности.

1. Introduction

One of the most rapidly developing fields of medico-biological studies originating from recent physico-chemical developments is the use of various organic and inorganic nanoparticles as agents for therapeutic purposes. Apart from their well-known application as drug carriers for controlled delivery, they are also used as intermediary agents in such novel medical treatment methods as photodynamic therapy (PDT) [1]. PDT is a promising method of cancer treatment based on administration of a photosensitizer — a specially designed substance, the molecules of which are accumulated in the tumor tissue and are further irradiated with light at the absorption wavelength. The excited photosensitizer molecules transfer the excitation energy to the molecular oxygen, generating reactive oxygen species (e.g., singlet oxygen) that are toxic to the tumor tissue. In various developments of PDT technologies, "intermediate" nanoparticles of different kinds are involved, either ensuring the irradiation conversion to the optimized wavelength range or facilitating the interaction of the involved components with biological tissues [2–9].

There have been certain considerations that PDT effects are largely concentrated on cell membranes, while the effects of radiotherapy — on nuclear DNA [10]. Thus, interaction of model phospholipid membranes with nanoparticles that can both absorb X-rays and generate singlet oxygen, as well as the effect of DNA on this interaction seemed an interesting subject for our studies.

The nanoparticles chosen for our study were either typical examples of biologically active nanosystems based on inorganic materials or objects originating from the living species but retaining characteristics allowing us to consider them as model nano-objects.

2. Materials and methods

Fullerene-containing silica nanoparticles (fullerene amino silica, SiO_2–C_{60}, containing 0.15 mmol C_{60} per 1 g SiO_2) as well as fullerene-containing silica nanoparticles with attached palladium atoms (SiO_2–C_{60}–Pd, containing 0.25 mmol Pd and 0.1065 mmol C_{60} per 1 g SiO_2) were synthesized as described in [11]. Deoxyribonucleic acid sodium salt from salmon testes (DNA), as well as low molecular weight deoxyribonucleic acid from salmon sperm (LmwDNA) were obtained from Sigma-Aldrich (USA).

Differential scanning calorimetry (DSC) studies were carried out using a Mettler DSC 1 microcalorimeter (Switzerland) with STAR^e software. The thermal scans were performed on heating and cooling at 2 K/min. The samples of 15–20 mg were placed into standard aluminum pans and sealed. Each sample was repeatedly scanned several times to achieve reproducibility.

Model phospholipid membranes of hydrated *L*-α-dipalmitoyl phosphatidyl choline (DPPC, Avanti Polar Lipids) were obtained in form of multilamellar vesicles. The DPPC to water ratio was 1:9. DPPC membranes without nanoparticles were taken as reference samples. To obtain DPPC membranes with nanoparticles, the following procedure was performed. All nanoparticles were dispersed in double distilled water using ultrasound bath. DPPC was hydrated by appropriate amount of water suspension of nanoparticles and pure water to ensure DPPC:water ratio as 1:9 and the required DPPC:nanoparticle ratio. The samples were stored for 120 h at room temperature with periodical heating up to 50–60°C and intensive agitation. The procedures of sample preparation were essentially similar to those described in our earlier papers [12, 13].

3. Results and discussion

Upon heating, DPPC membranes undergo the following sequence of phase transitions: a) pretransition from the low temperature gel phase to ripple phase (T_p ~ 36°C) and b) the main phase transition (T_m ~ 42°C) from the ripple phase to liquid-crystalline phase [14, 15]. Both phase transitions are of first order, and the corresponding peaks could be observed on the DSC thermograms.

The representative DSC scans of DPPC membranes containing gradually increasing concentrations of SiO_2–C_{60} are shown in Fig. 1. One can see that the main phase transition temperature T_m remains practically unchanged, and the peak shapes remain essentially similar. The same largely applies to the broader and less intensive pre-transition peaks (T_p). Similar behavior is observed upon cooling; though the measured Tm values are somewhat lower (by ~ 1.3°C) due to thermal hysteresis, but they are also practically independent on concentration.

A similar picture was observed with SiO_2–C_{60}–Pd; however, in this case the pretransition temperature T_p showed a slight decrease (at ~ 0.4°C) at higher concentrations (Fig. 2a), which could be ascribed to rearrangement of the phospholipid polar

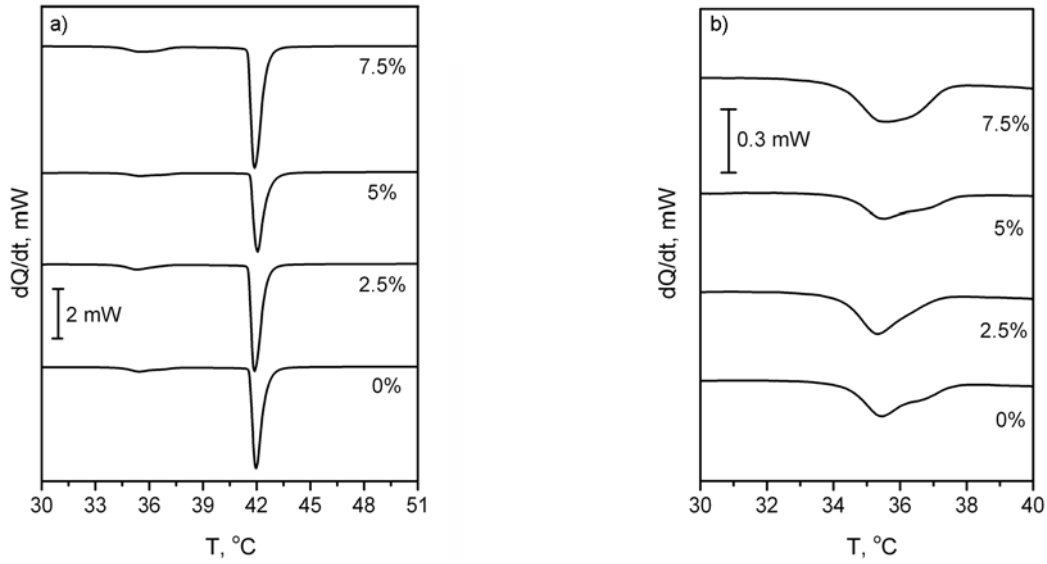

Fig. 1. DSC thermograms (heating) of DPPC membranes doped with SiO_2–C_{60}: the overall view (a); rescaled region of pre-transition peak (b). SiO_2–C_{60} weight concentrations with respect to dry DPPC are indicated.

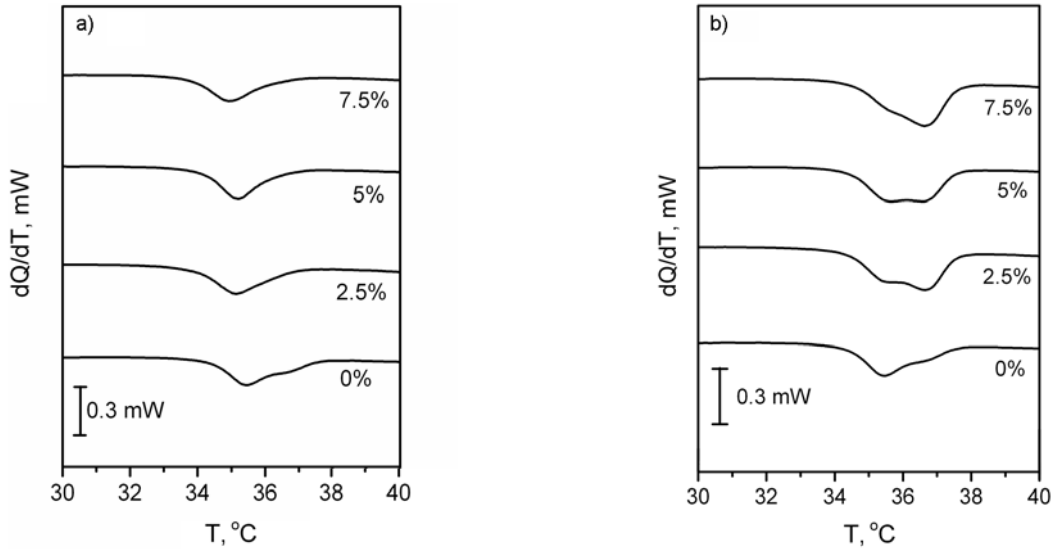

Fig. 2. DSC thermograms (heating) of the pre-transition region for DPPC membranes doped with SiO_2–C_{60}–Pd (a) and DNA (b). Dopant weight concentrations relatively dry DPPC are displayed.

heads due to interaction of the membrane surface with nanoparticles. Even more stable was T_p upon addition of DNA; however, a certain redistribution of the calorimetric signal over the broad pre-transition peak could be observed (Fig. 2b). In both cases, the position and the shape of the main phase transition peak remained stable within the concentration range under consideration.

Thus, the nanoparticles SiO_2–C_{60}, SiO_2–C_{60}–Pd and DNA under individual introduction practically do not affect the measured characteristics of DPPC membranes. Respectively, we can assume that they do not disturb the lipid order and molecular packing in the membrane. Such behavior suggests the absence of pronounced interaction of the nanoparticles SiO_2–C_{60}, SiO_2–C_{60}–Pd and DNA with DPPC membrane.

A qualitatively different behavior was observed for LmwDNA (Fig. 3). T_m is clearly rising with dopant concentration w, with an increase in dT_m/dw. Pronounced peak broadening with dramatic peak shape change (splitting) could be noted with increasing dopant content. It should be noted

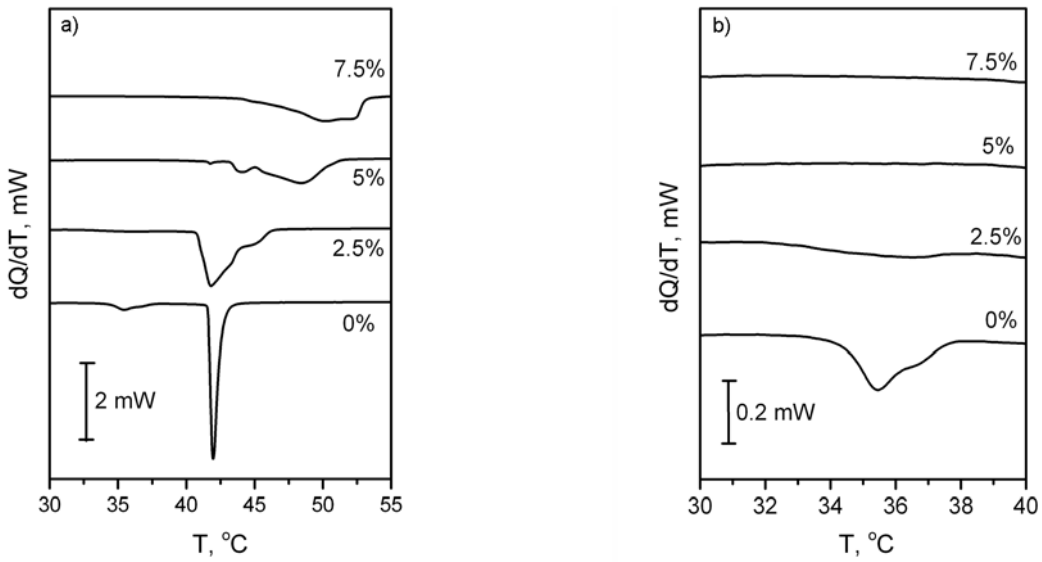

Fig. 3. DSC thermograms (heating) for DPPC membranes doped with LmwDNA: the whole range (a) and pre-transition region (b). LmwDNA weight concentrations with respect to dry DPPC are indicated.

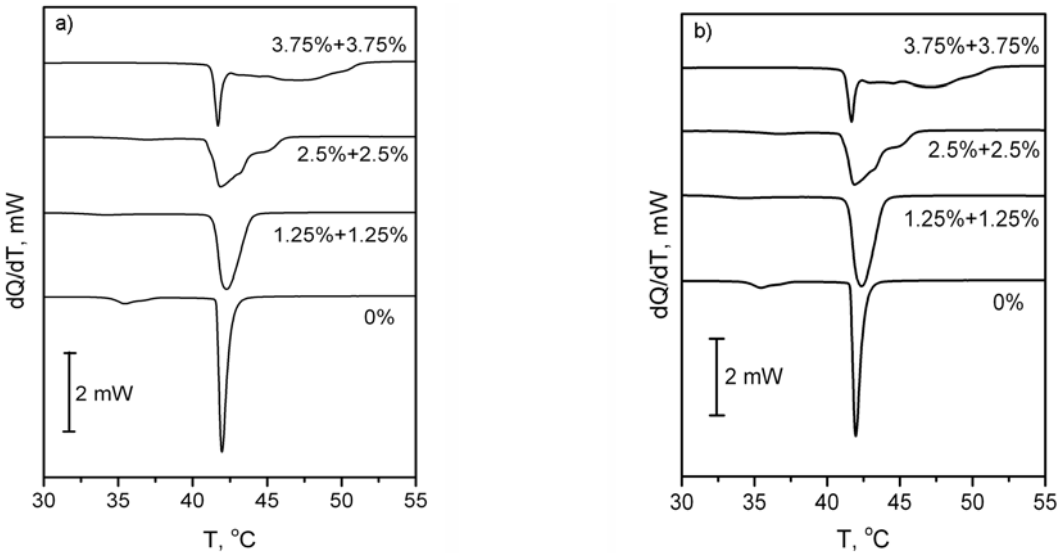

Fig. 4. DSC thermograms (heating) for DPPC membranes under joint addition of LmwDNA + SiO_2–C_{60} (a) and LmwDNA + SiO_2–C_{60}–Pd (b). Dopants were added in equal weight proportion, their weight concentrations with respect to dry DPPC are indicated.

that for the pre-transition at 2.5 % LmwDNA the peak was essentially blurred without substantial shift in the phase transition temperature. At higher concentrations of LmwDNA the pre-transition peak completely disappears. This suggests the presence of strong interaction between the LmwDNA and the DPPC membrane involving hydrophilic as well as hydrophobic regions of lipid membrane and resulting in decrease of the membrane homogeneity.

The next step was studing membranotropic effects of joint introduction (at equal weight proportion) of DNA with SiO_2–C_{60} (or SiO_2–C_{60}–Pd) and LmwDNA with SiO_2–C_{60} (or SiO_2–C_{60}–Pd). In the former case, joint addition of DNA + SiO_2–C_{60} (or DNA + SiO_2–C_{60}–Pd) has no essential effect on the phase transitions of DPPC membrane, as there were in the case with the individual components. As for joint addition of LmwDNA + SiO_2–C_{60} and LmwDNA + SiO_2–C_{60} in DPPC membrane, the DSC profiles were almost identical in these two cases and they noticeably change with

dopants concentration. But no new features at DSC profiles appear, as compared with effect of LmwDNA alone — the peaks present additive superposition, with the general shape determined by LmwDNA, Fig. 4a,b. Thus, in all cases no peculiarities of joint introduction of nanosystems (DNA + SiO_2–C_{60}, DNA + SiO_2–C_{60}–Pd, LmwDNA + SiO_2-C_{60} LmwDNA + SiO_2–C_{60}–Pd) into DPPC membrane were observed.

4. Conclusions

Interaction of nanosystems SiO_2–C_{60}, SiO_2–C_{60}–Pd, DNA and LmwDNA with DPPC model membrane were studied by DSC technique.

It was found that SiO_2–C_{60}, SiO_2–C_{60}–Pd and DNA could be considered as "inert" to the membrane because they have almost no effect on DPPC membrane phase transitions.

LmwDNA has pronounced effect on DSC profiles (both pre-transition and main phase transition) which increases with nanosystem concentration. The effect of LmwDNA is characterised by increasing of the main phase transition temperature, significant peak broadening and splitting and vanishing of the pre-transition peak.

No peculiar features (such as deviations from additivity) under joint introduction of the nanosystems to DPPC membrane could be noted.

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References

1. W.Chen, J.Zhang, *J.Nanosci.Nanotech.*, **6**, 1159 (2006).
2. W.Sun, Z.Zhou, G.Pratx et al., *Theranostics*, **10**, 1296 (2020).
3. A.-L.Bulin, C.Truillet, R.Chouikrat et al., *J. Phys.Chem.C.* **117**, 21583 (2013).
4. X.Zou, M.Yao, L.Ma et al., *Nanomedicine.* **9**, 2339 (2014).
5. S.Kascakova, A.Giuliani, S.Lacerda et al., *Nano Research.*, **8**, 2373 (2015).
6. H.Chen, G.D.Wang, Y.J.Chuang et al., *NanoLett.*, **15**, 2249 (2015).
7. D.R.Cooper, K.Kudinov, P.Tyagi et al., *Phys. Chem.Chem.Phys.*, **16**, 12441 (2014).
8. Clement, W.Deng, E.Camilleri et al., *Scie. Reports*, **6**, 19954 (2016).
9. S.L.Yefimova, T.N.Tkacheva, P.O.Maksimchuk et al., *J.Luminescence,* **192**, 975 (2017).
10. J.Xu, J.Gao, Q.Wei, *J.Nanomater.*, **2016**, 8507924 (2016).
11. M.O.Davydenko, E.O.Radchenko, V.M.Yashchuk et al., *J.Molec. Liq.,* **127**, 145 (2006).
12. O.Vashchenko, V.Pashynska, M.Kosevich et al., *Mol.Cryst.Liq.Cryst.*, **547**, 155 (2011).
13. N.A.Kasian, V.A.Pashynska, O.V.Vashchenko et al., *Mol.BioSyst.*, **10**, 3155 (2014).
14. R.Koynova, M.Caffrey, *Biochim.Biophys. Acta*, **1376**, 91 (1996).
15. L.N.Lisetski, O.V.Vashchenko. N.A.Kasian, L.V.Sviechnikova, *Springer Proc. Phys*, **266**, 85 (2021).