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Self-assembly of interpolyelectrolyte complexes and mixed micelles from guanidinium and phosphonate derivatives of *p–tert–*butylthiacalix[4]arene and solubilization of paclitaxel

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ABSTRACT

In this work, supramolecular assemblies based on *p–tert–*butylthiacalix[4]arene derivatives functionalized with charged guanidinium and phosphonate fragments in the *1,3–alternate* conformation were obtained in the presence of the surfactants. Two main approaches based on the macrocyclic compounds have been presented for supramolecular assembling, i.e. (i) the use of two oppositely charged macrocyclic compounds forming interpolyelectrolyte complexes; and (ii) the use of ionogenic macrocycles and low molecular surfactants forming mixed micelles. Self-assembling properties of guanidinium and phosphonate derivatives of *p–tert–*butylthiacalix[4]arene and paclitaxel solubilization of interpolyelectrolyte complexes and mixed micelles were examined and compared for various compositions. The obtained supramolecular associates were further investigated to load hydrophobic anticancer drug paclitaxel that is insoluble in water and requires solubilization prior to application. It was established that mixed micelles containing thiacalix[4]arene with guanidinium fragments were more effective in solubilizing paclitaxel against interpolyelectrolyte approach. The micelles obtained in the presence of water-insoluble drug formed stable monodisperse colloidal system consisted of spherical particles ($d = 192 \pm 7$ nm, $\zeta = 33 \pm 4$ mV for equimolar ratio of the components). The results offer a new strategy for the design of the interpolyelectrolyte complexes based on the macrocyclic building blocks for the solubilization of water-insoluble drugs.

Engineering of nanoparticles capable of encapsulating water-insoluble active substances attracts increasing attention of researchers. A large number of active compounds important for the therapy against tumor diseases are poorly soluble in water. This makes them unsuitable for further drug development because of the difficulties associated with their targeted delivery [1,2]. For this reason, search and creation of nanoparticles as carriers of water–insoluble drugs has become an important area of nanotechnology [3,4].

Several strategies have been previously used to create drug nanoparticles, e.g., nanoemulsions [5,6], nanosuspensions [7], solid lipid nanoparticles [8], liposomes [9], micelles [10], and inorganic nanoparticles [11]. The use of interpolyelectrolyte complexes (IPEC) is one of the promising approaches intended to increase the drug solubility, reduce their untimely oxidation and suppress nonspecific interactions. IPECs [12–16] belong to a class of the substances formed by the combination of charged polyelectrolytes involved in reversible reaction with oppositely charged polyions [17]. Cooperative character of bonding between the polyions provides high stability of the IPECs in a wide pH range of the medium. Anionic and cationic macromolecules are electrostatically complementary to each other. Therefore, interactions between them are similar to those of complementary biopolymers, including self-assembly, from the thermodynamic point of view.

The use of macrocyclic compounds is a promising direction to the development of IPECs. This makes it possible to obtain materials with a certain structure and properties yielding from the presence of macrocyclic cavity and substituents attached to it [18–20]. The interaction of the oppositely charged macrocyclic polyelectrolytes leads to changes in the properties of the supramolecular complexes caused by cooperative aggregation of the components [21,22]. Thiacalix[4]arene derivatives are a convenient platform for creating such structures. Thiacalix[4]arene has special advantages over "classical" calix[4]arene [23]. Due to the larger volume of the sulfur atom and, accordingly, the lengthening of

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the Ar-S-Ar bond, the ease of formation of stereoisomeric forms increases, when passing from the "classical" to thiacalix[4]arene. Replacing the methylene bridge with sulfur atoms made it possible to obtain four stereoisomeric forms (*cone*, *partial cone*, *1,2-alternate*, *1,3-alternate*) in good yields. In the case of calix[4]arene, only the *cone* conformation is obtained with good yield. It is known that biological activity depends on the conformation of thiacalix[4]arene [23].

Thiacalix[4]arenes provide conformationally flexible molecular platform capable of forming miscellaneous types of structures, which are different in morphology and composition and applied in many areas [23,24]. Variation of the dimensions of the internal cavity, even within certain limits, promotes changes in the number and nature of the binding sites and in the macrocycle conformation. To date, literature presents a single approach to the formation of IPECs, i.e., formation of the complex between two oppositely charged polymers [13–16,25]. This became possible due to the ability of the macrocycles to self-assemble into supramolecular associates. Functionalization of the macrocycles with charged fragments together with their ability to self-assemble into the supramolecular associates facilitates obtaining the IPEC analogs based on the polymers [21]. The introduction of guanidinium and aminobis(methylenephosphonic acid) fragments into the macrocycle structure contributes to the manifestation of specific properties. These functional groups show anchoring properties for various types of biomolecules and substrates and exert high affinity toward nucleic acids and metal ions [26–28]. The literature describesthe use of these fragments for the functionalization of various macrocycles [29] and natural polymers [30] and the study on their biological activity, sorption and catalytic properties. Only few articles describe the use of aminophosphonic and guanidinium fragments in the composition of metacyclophanes for molecular programming and creation of a constructor for the formation of complex supramolecular assemblies.

Thereby, we propose in this work two main approaches to the creation of supramolecular assemblies based on macrocyclic compounds: (i) the use of two oppositely charged macrocyclic compounds forming IPECs; and (ii) the use of ionogenic macrocycles and low molecular surfactants forming mixed micelles (Fig. 1). Mixed micelles based on the macrocycles are extensively studied and widely used [20]. The use of *1,3–alternate* conformation of the *p–tert*–butylthiacalix[4]arene in the creation of IPEC favors changes in the surface charge of the micelles by the arrangement of charged fragments on both sides of the macrocyclic ring [21]. Thus, it leads to an increase in the stability of the system.

Paclitaxel is a cytostatic anticancer drug that belongs to the taxanes family. It is one of the most used herbal anticancer drugs. Paclitaxel is used in the treatment of the cancer of the ovaries, breast, lung, cervix, pancreas, and Kaposi's sarcoma [31–34]. Despite high efficiency, paclitaxel is poorly soluble in water and this limits its scope. Thus, present work is devoted to the preparation of the IPECs based on the thiacalix[4]arene derivatives in *1,3–alternate* conformation containing charged fragments and surfactants and to the study of their solubilizing ability with respect to paclitaxel.

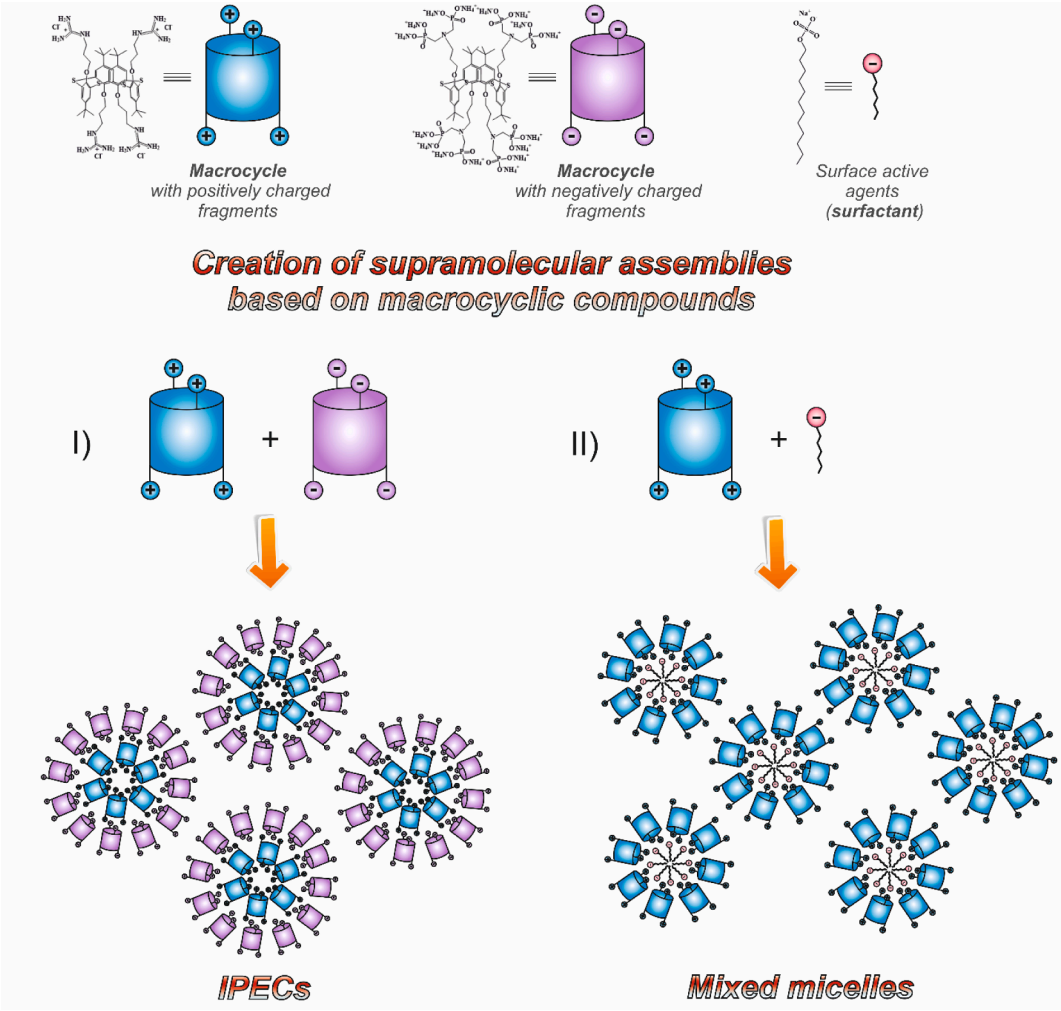

Fig. 1. Schematic representation of two main approaches to the creation of supramolecular assemblies based on macrocyclic compounds.

1. Materials and methods

Most of the reagents were purchased from Aldrich and used without further purification. All the aqueous solutions were prepared with the Millipore–Q deionized water (>18.0 MΩ × cm at 25 °C).

Thiacalix[4]arenes (**1–3**) were synthesized by the literature method [35,36].

1.1. Dynamic light scattering (DLS)

Particle size. The particle size was determined by the Zetasizer Nano ZS instrument at 20 °C. The instrument contains 4 mW He–Ne laser operating at a wavelength of 633 nm and incorporated noninvasive backscatter optics (NIBS). Measurements were performed at the detection angle of 173° and the software automatically determined the measurement position within the quartz cuvette. Experiments were carried out for each solution in triplicate. Synthesized thiacalix[4]arenes **1–3** were dissolved in water at the concentrations used in further research. Studies on aggregation of thiacalix[5]arenes **1–3** in water in various ratios, in the presence or absence of the surfactants (sodium dodecyl sulfate (**SDS**) or dodecyltrimethylammonium chloride (**DTAC**)), and drug (paclitaxel) were carried out in the concentration range from 5 × 10^{-7} M to 1 × 10^{-4} M. Dry sample of the drug was poured with the appropriate solution of mixed particles of the macrocycle **–** surfactant or IPEC, then the solution was kept in the ultrasonic bath for 20 min. and thermostated at room temperature. After that, the size of the particles formed was measured.

Zeta Potentials. Zeta potentials were measured on the Zetasizer Nano ZS from Malvern Instruments. Samples were prepared as for the DLS measurements and transferred with the syringe to the disposable folded capillary cell. The zeta potential was measured using the Malvern M3–PALS method, and reported values were taken from the average of three measurements.

1.2. UV–visible spectroscopy

Absorption spectra were recorded on the Shimadzu UV–3600 spectrometer (Kyoto, Japan). Quartz cuvettes with an optical path length of 10 mm at 25 °C were used. Water was used for preparation of the solutions. The absorption spectra of the thiacalix[4]arene **3** (5 × 10^{-5} M), **SDS**, paclitaxel, and their mixtures in equimolar ratio were recorded after thermosetting the solutions for 15 min. at 25 °C.

1.3. Transmission electron microscopy (TEM)

TEM analysis of the particles based on synthesized macrocycles **1–3** (particles macrocycle-surfactant, IPEC [**2** + **3**] + **Paclitaxel**, and [**3** + **SDS**] + **Paclitaxel**) was carried out using the Hitachi HT7700 Exalens transmission electron microscope with Oxford Instruments X–Max^n 80 T EDS detector. The samples were prepared in the same way as for the DLS experiments. For the sample preparation, 10 μL of the suspension were placed on the Formvar™/carbon coated 3 mm copper grid, which was then dried at room temperature. After complete drying, the grid was placed into the transmission electron microscope using special holder for microanalysis. Analysis was hold at the accelerating voltage of 80 kV in the STEM mode using Oxford Instruments X–Max^n 80 T EDS detector.

1.4. Encapsulation efficiency

The encapsulation efficiency (EE, %) of paclitaxel into particles formed by [**3** + **SDS**] was calculated using the following equation:

$$EE = \frac{Total\ amount\ of\ paclitaxel - free\ paclitaxel}{Total\ amount\ of\ paclitaxel} \times 100\%$$

Appropriate parameters were determined indirectly by centrifugation. The amount of free substrate was determined using spectrophotometric method. Centrifuge was used to separate associates with encapsulated paclitaxel from free paclitaxel. Associates with encapsulated paclitaxel were placed in the centrifuge tube and centrifuged at 10,000 rpm for 10 min. After centrifugation, free substrate in the aqueous solution went to the bottom of the centrifuge tube. The volume of the solution from the lower phase was accurately measured, and then its absorption spectra were recorded. The concentration and mass of free compound were calculated from the absorption spectra and extinction coefficient and then used to calculate encapsulation efficiency and loading efficiency.

2. Results and discussions

2.1. Self–assembly of the water–soluble macrocycles and surfactants into the mixed interpolyelectrolyte nanoparticles

Surfactants, macrocycles and amphiphilic polymers are mostly used for solubilizing poorly water-soluble compounds including drugs [37–40]. For the synthesis of mixed interpolyelectrolyte nanoparticles, thiacalix[4]arenes **1–3** containing phosphonate and guanidinium groups (Fig. 2) were chosen together with the surfactants (**SDS** and **DTAC**). At the first stage of the work, self–assembly of the synthesized water–soluble thiacalixarenes **1–3** in the *1,3–alternate* conformation and of the surfactants was studied in water.

Two–component macrocycle/surfactant systems were studied using DLS to determine hydrodynamic diameter of the particles formed, polydispersity index (PDI) and to assess the charge and stability of the systems (Table 1).

In all the studied **2:DTAC** ratios, no formation of colloidal systems with low PDI and unimodal size distribution by intensity was observed. The particle diameter varied from 196 to 528 nm (PDI = 0.54 ÷ 0.62). Probably, this could be due to electrostatic repulsion of the quaternary ammonium ions present in the structure of the macrocycle and of the surfactant. In addition, possible replacement of a NH_4^+ cation of **2** by alkylammonium cation of **DTAC** would also destabilize the system.

A unimodal system in the case of positively charged **DTAC** added to the macrocycle **1** was observed only for their ratio of 1:4 (Fig. 3). For other ratios of thiacalix[4]arene **1** + **DTAC**, the systems with low PDI have not been found. In this case, it can be assumed that the system with unimodal particle intensity distribution is achieved mainly due to the electrostatic compensation of the charges of phosphonate groups in the structure of the macrocycle **1** by oppositely charged trimethylammonium fragments of **DTAC**. Perhaps, zwitterionic form of the macrocycle **1** prevails by migration of the proton from the hydroxyl group to the lone electron pair at the nitrogen atom. This process is dynamic and results in the formation of polydisperse and unstable system. Colloidal systems of all types of the particles based on the macrocycle **1** are unstable as follows from the ζ–potential values (Table 1).

The equimolar system of the macrocycle **3** and **SDS** exerts molar excess of the guanidinium fragments of the thiacalix[4]arene against sulfo groups in the surfactant molecule. According to the ^{1}H NMR spectroscopy, no significant changes in the spectrum of the **3** + **SDS** in the 1:1 M ratio were observed (Fig. S54). Hence, no host–guest complex was formed, but the formation of associates took place as follows from the DLS and TEM data (Fig. 4). The ζ-potential value also coincides with the formation of an extremely stable system (51 ± 4 mV). Further increase in the concentration of the surfactant leads to a higher PDI and destabilization of the system. The TEM micrograph of the **3** + **SDS** presented in Fig. 4B showed that the macrocycle **3** was capable of forming mixed associates with the **SDS** that had near-spherical shape with a narrow size distribution.

The surface of the particles underwent rearrangement and recharging for the **3** + **SDS** system in the 1:4 M ratio (ζ = –40 ± 4 mV, Table 1). It can be assumed that the structure of the particles allowed electrostatic interaction of the charged fragments of the molecules with each other. The IPEC surface charge depends on the strength of the acid and base

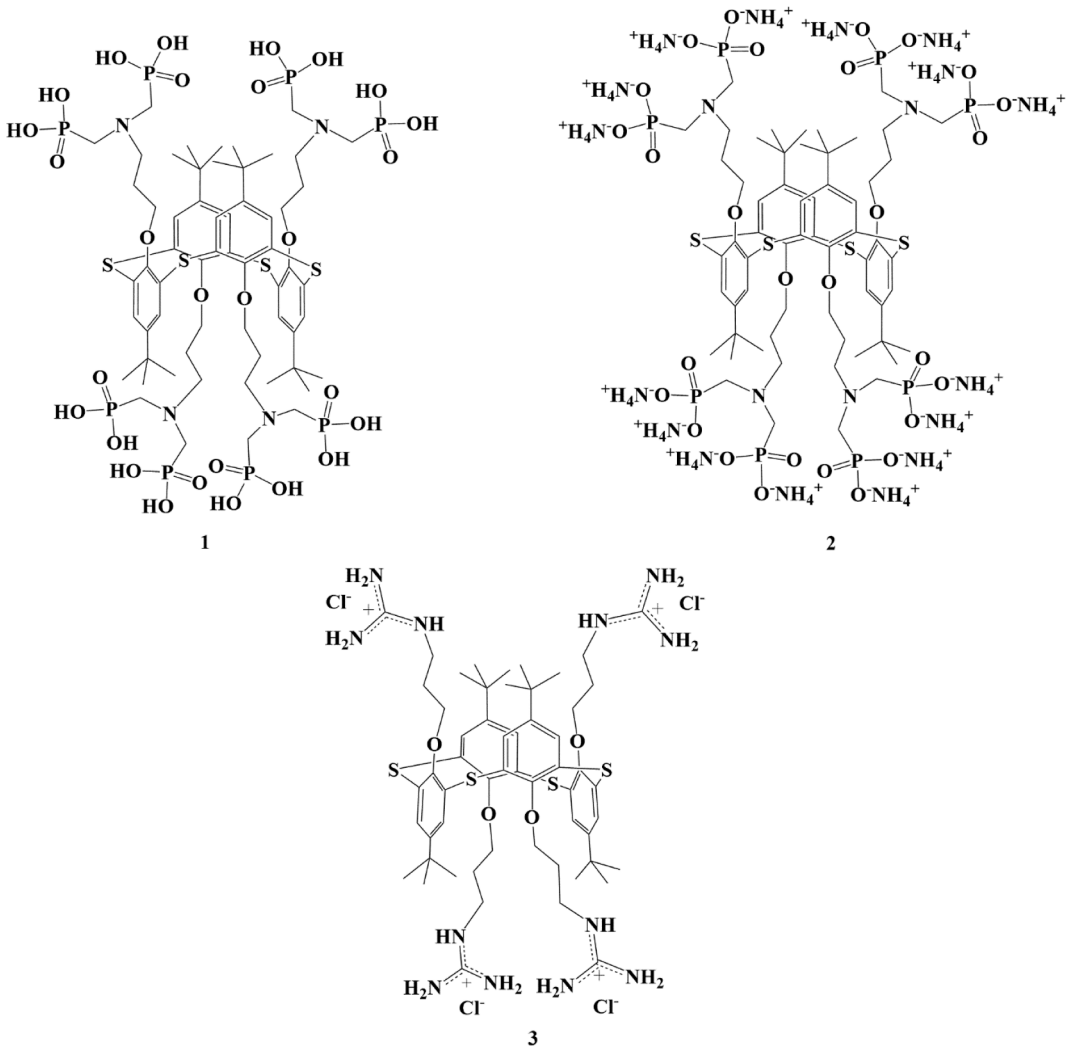

Fig. 2. Structures of *p–tert*–butylthiacalix[4]arene derivatives **1–3** containing phosphonate and guanidinium fragments in the *1,3–alternate* conformation.

Table 1
Aggregate size (hydrodynamic particle diameter, d, nm), intensity distribution of mixed particles formed in the association of the compounds **1**, **2** and **3** in water with the different surfactants (**SDS** and **DTAC**); polydispersity index (PDI) and zeta potential (ζ) at different macrocycle-surfactant ratio.

Macrocycle: Surfactant ratio	**1 + DTAC**			**2 + DTAC**			**3 + SDS**		
	PDI	d, nm	ζ, mV	PDI	d, nm	ζ, mV	PDI	d, nm	ζ, mV
1:1	0.76 ± 0.18	424 ± 93 (71 %) 74 ± 19 (29 %)	−12 ± 3	0.54 ± 0.06	425 ± 126 (65 %) 135 ± 44 (35 %)	−36 ± 2	**0.13 ± 0.01**	**99 ± 2**	**+51 ± 4**
1:4	**0.20 ± 0.04**	**180 ± 6**	**−3 ± 1**	0.62 ± 0.06	196 ± 78 (58 %) 705 ± 219 (31 %)	−34 ± 2	0.25 ± 0.02	40 ± 1 (89 %)	−40 ± 4
1:8	0.46 ± 0.04	327 ± 65 (71 %) 67 ± 11 (29 %)	−3 ± 2	0.58 ± 0.09	424 ± 88 (90 %) 54 ± 8 (10 %)	−20 ± 1	0.50 ± 0.01	36 ± 1 (70 %)	−40 ± 4
1:50	0.46 ± 0.04	836 ± 167	−3 ± 0	0.60 ± 0.10	528 ± 185 (79 %) 118 ± 30 (17 %)	−61 ± 5	0.54 ± 0.14	26 ± 16 (53 %) 283 ± 82 (47 %)	−38 ± 2

(their dissociation degree). For the equimolar ratio of the guanidinium fragments and of the sulfonate groups, the surface was negatively charged. In this case, different number of cations and anions is formed in dissociation and there is no complete compensation of the charges as follows from the negative zeta potential of the IPEC surface. Probably, the macrocycle **3** is incorporated into the micelles formed by **SDS** (Fig. 5).

It was impossible to reveal patterns of changes in the particle diameter and polydispersity indexes of systems based on the macrocycles **1**–**3** and **SDS** or **DTAC**.

2.2. Self–assembly of [1 + 3] and [2 + 3] interpolyelectrolyte aggregates with surfactants (SDS and DTAC) into mixed interpolyelectrolyte nanoparticles

One of the essential tasks for modern supramolecular chemistry is the stabilization of the systems for solving problems related to encapsulation and subsequent targeted delivery of the drugs, diagnosing markers, and to design of highly effective receptors for various biological substrates [41–43].

It is known that surfactants increase stability of disperse systems and aggregation stability of existing dispersions. Recently, surfactants have

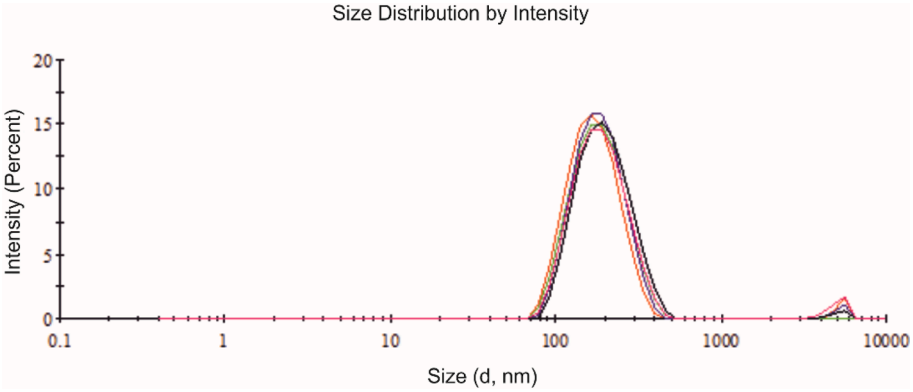

Fig. 3. Size distribution of the macrocycle **1** (1×10^{-4} M) in the presence of **DTAC** in the 1:4 M ratio in an aqueous solution.

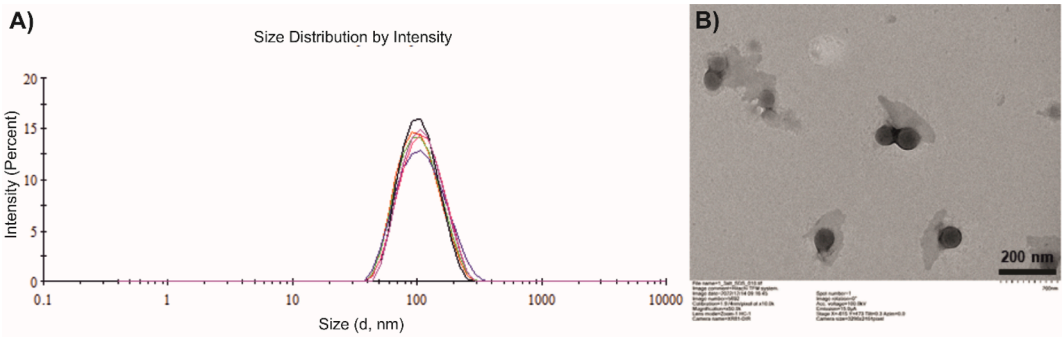

Fig. 4. (A) Size distribution and (B) TEM image of the particles formed by the macrocycle **3** (1×10^{-4} M) and **SDS** in a 1:1 M ratio.

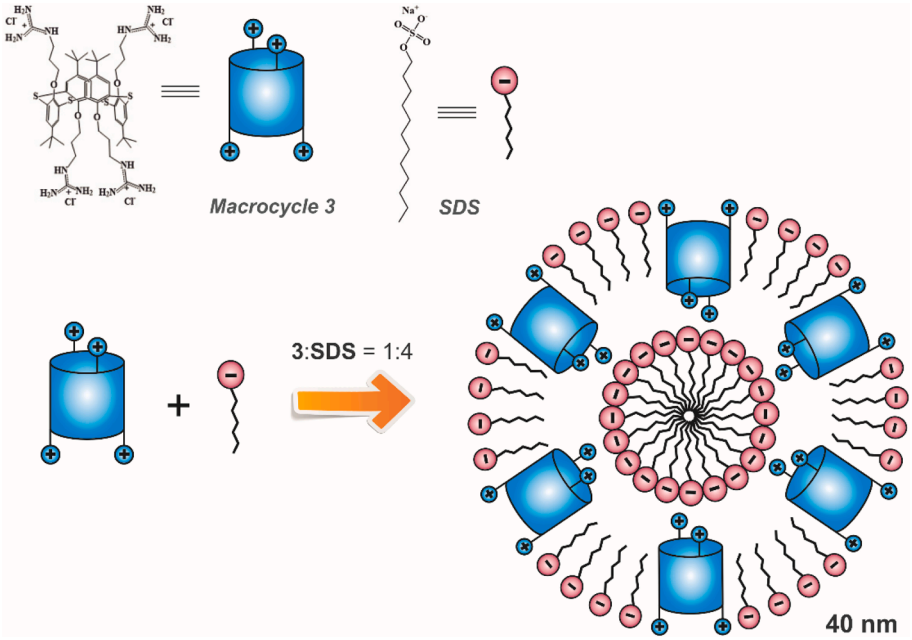

Fig. 5. Schematic representation of the particle formation from macrocycle **3** (1×10^{-4} M) and **SDS** in a 1:4 M ratio.

found application also for stabilization of the macrocyclic systems based on pillar[5]arenes and (thia)calix[4]arenes [12,44].

Previously, our research team showed that the macrocycles **1**–**3** were capable of self–assembling through instantaneous ion exchange between the oppositely charged macrocycles resulting in formation of the IPEC **[1 + 3]** and **[2 + 3]**. In the case of IPECs **[1 + 3]**, the systems showed the highest stability for the macrocycle concentration of 5×10^{-7} M and the lowest one for 5×10^{-5} M. The **[2 + 3]** based system demonstrated opposite direction of the changes [45].

In this regard, we have made attempts to stabilize the least stable IPEC **[1 + 3]** and **[2 + 3]** by adding surfactants to obtain colloidal systems with the unimodal particle distribution. Various molar macrocycle-surfactant ratios (1:1, 1:100, and 1:1000) were selected to study the effect of the surfactant molecules on the IPEC.

The IPEC **[1 + 3]** (5×10^{-5} M) was characterized by the PDI (0.50 ± 0.30) and hydrodynamic particle diameter of 2266 ± 685 nm [45]. Based on our data presented earlier, the ζ potential of the particles formed by the macrocycles **1** and **3** was negative. In this regard, it was logical to add positively charged **DTAC** molecules to stabilize the initial particles by electrostatic interactions. The PDI values and hydrodynamic particle diameters decreased with increased macrocycle-surfactant ratio for the **[1 + 3] + DTAC** system (Table 2) reaching particle size of 1749 ± 137 nm (PDI = 0.40 ± 0.04) for the macrocycle: surfactant ratio of 1:1000. The ζ potential regularly changed with the quantities of the surfactant added.

Stabilization of the systems was expected mainly due to the hydrophobic effect in the case of **[1 + 3] + SDS**, where the **1 + 3** associates and **SDS** molecules turn out to be negatively charged. In this series of reactant ratios, the system had the lowest PDI = 0.29 ± 0.01 with the molar ratio of macrocycle-surfactant = 1:100. However, it was not possible to identify patterns of changes in the hydrodynamic diameters of the particles and polydispersity indexes for these systems. It should be noted that the surface of **[1 + 3] + SDS** associates turns out to be negatively charged, and this system is more stable than **[1 + 3] + DTAC**. No systems with low polydispersity indexes based on IPECs **[1 + 3]** were observed both in the case of the addition of **DTAC** and **SDS**. Probably, this is due to the fact that IPEC **[1 + 3]** is formed by the thiacalixarene **1** containing residues of aminobis(methylenephosphonic acid), which is not completely dissociated in water. As a result, the formation of stable monodisperse systems was not observed.

While studying the systems based on the IPEC **[2 + 3]** with **DTAC**, no stable nanoparticles were obtained for all the studied ratios (Table 3). The hydrodynamic diameters of the obtained particles ranged from 209 to 524 nm. In addition, the zeta potential of the system significantly decreased with an increase in the **DTAC** ratio. This indicates its instability. Probably, the addition of **DTAC** to IPEC **[2 + 3]** led to its destruction. Here, the competitive formation of the mixed micelles **[2 + DTAC]** is possible due to electrostatic interaction. The IPECs **[2 + 3]** were characterized by negative values of the zeta potential [45]. In our previous work, we showed that decrease in the concentration of the solutions **[2 + 3]** led to the destabilization of the system and to the growth of its polydispersity. In the case of mixed **[2 + 3] + SDS** particles, decrease in polydispersity indexes and increase in the stability of corresponding systems were observed with increased **SDS** concentration (Table 3).

Probably, rearrangement of the mixed micelles takes place. First, the macrocycle **3** with positively charged fragments adheres to the **SDS** micelle due electrostatic attraction. Then, the macrocycle **2** with negatively charged fragments joins them. A significant **SDS** excess completes the mixed micelle to the stable monodisperse system as a "molecular glue" (Fig. 6C). Thus, stable system was obtained with the 1000–fold excess of the surfactant against the macrocycle with the particle size of about 154 nm (PDI = 0.28 ± 0.04) (Fig. 6A).

Additionally, the colloidal stability of the supramolecular self-assemblies **3 + SDS** and **[2 + 3] + SDS** was evaluated in biorelevant conditions, i.e., in saline and Ringer-Locke's solution (Table 4). It was shown that the unimodal distribution of nanoparticles and the zeta potentials retained both in saline and Ringer-Locke's solution, while the particle size did not. Probably, saline and Ringer-Locke's solution decreased the nanoparticle size due to the salting effect. Nevertheless, it can be unequivocally stated that the presence of salts and components of Ringer-Locke solution did not affect the stability and monodispersity of the system.

Thus, using the DLS method, the formation of mixed micelles based on thiacalix[4]arenes **1**–**3** functionalized with guanidinium and phosphonate fragments, and on the surfactants (**DTAC** and **SDS**) was shown.

Table 2
Aggregate size (hydrodynamic particle diameters d, nm), intensity distribution of mixed particles formed by self–assembly of interpolyelectrolyte aggregate **[1 + 3]** in water with various surfactants (**SDS** and **DTAC**); polydispersity index (PDI) and zeta potential (ζ) at different macrocycle-surfactant ratios. $C_{macrocycle} = 5 \times 10^{-5}$ M.

Macrocycle-Surfactant ratio	**[1 + 3] + DTAC**			**[1 + 3] + SDS**		
	PDI	d, nm	ζ, mV	PDI	d, nm	ζ, mV
1:1	0.67 ± 0.10	5416 ± 1442	10 ± 3	0.62 ± 0.11	1161 ± 623	−15 ± 1
1:100	0.50 ± 0.10	3137 ± 229	23 ± 1	0.29 ± 0.01	1045 ± 73	−47 ± 1
1:1000	0.40 ± 0.04	1749 ± 137	34 ± 3	0.85 ± 0.08	1228 ± 359	−52 ± 2

Table 3
Aggregate size (hydrodynamic particle diameters d, nm), intensity distribution of mixed particles formed by self–assembly of the interpolyelectrolyte aggregate **[2 + 3]** in water with various surfactants (**SDS** and **DTAC**); polydispersity index (PDI) and zeta potential (ζ) at different macrocycle - surfactant ratios. $C_{macrocycle} = 5 \times 10^{-5}$ M.

Macrocycle: Surfactant ratio	**[2 + 3] + DTAC**			**[2 + 3] + SDS**		
	PDI	d, nm	ζ, mV	PDI	d, nm	ζ, mV
1:1	0.39 ± 0.01	209 ± 4	−18 ± 2	0.48 ± 0.06	226 ± 9	−18 ± 2
1:100	0.45 ± 0.05	304 ± 17	−5 ± 1	0.34 ± 0.02	159 ± 5	−29 ± 2
1:1000	0.30 ± 0.02	524 ± 56	−3 ± 1	**0.28 ± 0.04**	**154 ± 3**	**−37 ± 3**

2.3. Solubilization of paclitaxel with mixed IPECs based on thiacalix[4]arenes and surfactants

Synthesized nanoparticles different in diameter, surface charge, and stability can be loaded with various targeted therapeutic agents or fluorescent markers.

Based on the above, solubilizing ability of interpolyelectrolyte mixed particles has been studied for paclitaxel widely used as cytostatic anticancer drug. For this purpose, colloidal systems with unimodal distribution of the particles and low PDI index were selected. The macrocycle **1** and **DTAC** in molar ratio of 1:4, macrocycle **3 + SDS** (1:1) and stable mixed associates **[2 + 3] + SDS** were tested. Paclitaxel is insoluble in water, but the idea of drug solubilization assumes incorporation of the molecules into the hydrophobic region of the micelle-like systems.

The study of the interaction between binary systems (macrocycle–surfactant) and paclitaxel was carried out for two macrocycle-drug ratios, i.e., equimolar (1:1) and excessive (1:10) (Table 5). It was impossible to evaluate the parameters of colloidal systems by the DLS method for the excess of paclitaxel because of the formation of opaque solutions.

The **[1 + DTAC]** and **[2 + 3] + SDS** systems with paclitaxel showed high polydispersity (Table 5). In the case of the interaction of paclitaxel with mixed particles **[3 + SDS]**, fairly stable monodisperse systems were obtained as evidenced from the zeta potential (ζ = 33 ± 4 mV). Hydrodynamic diameter of the particles was equal to 192 ± 7 nm (PDI = 0.19 ± 0.04) (Fig. 7A). Zeta potential is often used to indicate the stability of the particle systems. In case of high ζ value, the particles repulse each other. This prevents their aggregation and results in enhanced stability of the solution. The resultant ζ values of **[3 + SDS]** and paclitaxel-loaded **[3 + SDS]** associates were equal to + 51 ± 4 mV and + 33 ± 4 mV, respectively. High positive potential was attributed to the ionized guanidinium groups of the macrocycle **3**. It was reported that positive charge of the particles could promote their endocytosis by cells [46]. It should be noted that no monodisperse particles were obtained in the reaction of **SDS** taken alone without macrocycles with paclitaxel (ratios 1:1 and 1000:1, see SI, Fig. S55-56). This confirms the preorganization role of the macrocycle **3** in the **[3 + SDS] + Paclitaxel** system (Table 5). Particles **[3 + SDS]** were capable to load 87 ± 2 % of

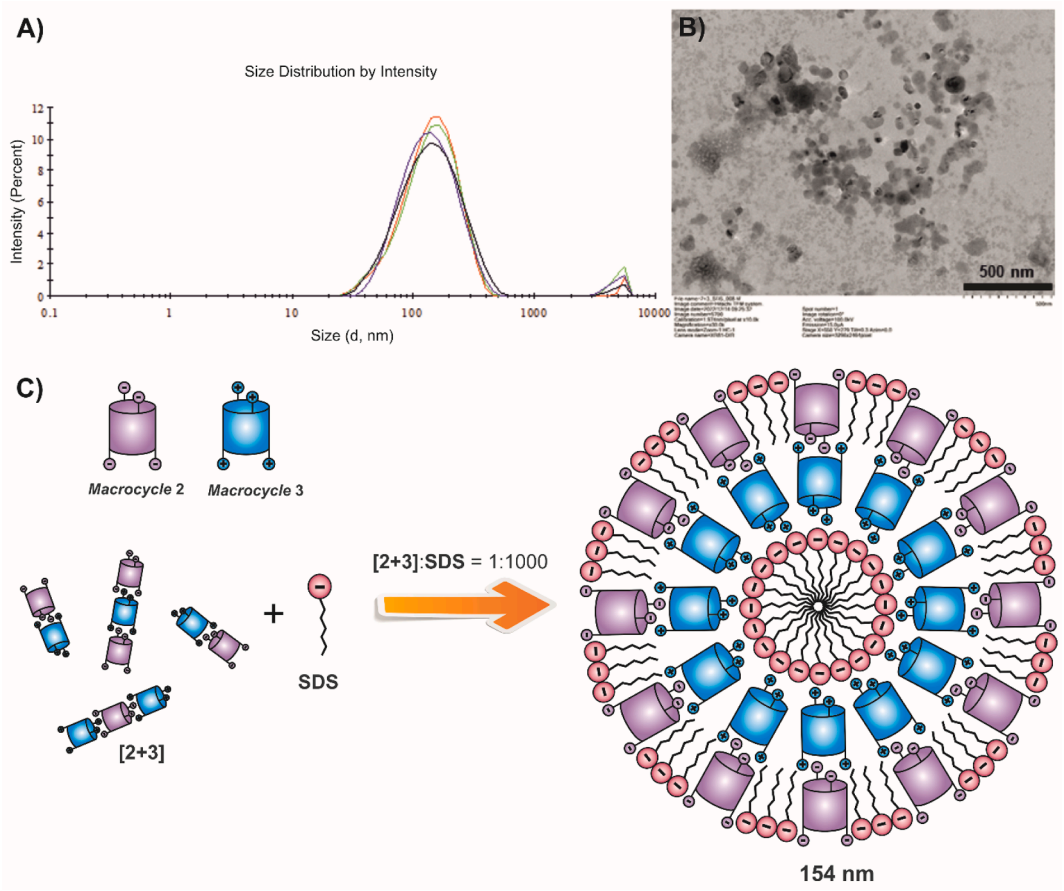

Fig. 6. (A) Size distribution, (B) TEM image and (C) schematic representation of the particles formed by IPEC **[2 + 3]** ($C = 5 \times 10^{-7}$ M) with **SDS** in a 1:1000 M ratio.

Table 4
Hydrodynamic diameter, polydispersity index (PDI) and zeta potential (ζ) of mixed particles formed by self–assembly of the macrocycle **3** and inter-polyelectrolyte aggregate **[2 + 3]** with the surfactant **SDS** at 1:1 and 1:1000 M ratio, correspondingly, in saline and Ringer-Locke's solution (298 K).

System (molar ratio)	Saline			Ringer-Locke's solution		
	d, nm	PDI	ζ, mV	d, nm	PDI	ζ, mV
3 + SDS (1:1)	76 ± 5	0.16 ± 0.02	+49 ± 1	76 ± 5	0.22 ± 0.04	+50 ± 3
[2 + 3] + SDS (1:1000)	109 ± 6	0.23 ± 0.05	−36 ± 3	109 ± 6	0.27 ± 0.06	−35 ± 4

Table 5
Aggregate size (hydrodynamic particle diameters d, nm), intensity distribution of mixed particles formed by association of mixed particles **1 + DTAC** (1:4), **3 + SDS** (1:1), **[2 + 3] + SDS** (1:1000) and paclitaxel and **SDS + Paclitaxel** (1000:1) in water, polydispersity index (PDI) and zeta potential (ζ).

System	PDI	d, nm	ζ, mV
[1 + DTAC] + Paclitaxel	0.74 ± 0.16	4503 ± 2861	−10 ± 1
[3 + SDS] + Paclitaxel	**0.19 ± 0.04**	**192 ± 7**	**+33 ± 4**
([2 + 3] + SDS) + Paclitaxel	0.53 ± 0.14	1171 ± 245	−34 ± 2
SDS + Paclitaxel	0.72 ± 0.13	395 ± 26	−28 ± 4

the total amount of paclitaxel added to the system.

TEM allowed direct visualization of the size and morphology of the micelles (Fig. 7B). The TEM micrograph of the paclitaxel-loaded associates showed that the **[3 + SDS]** formed associates of extended architecture consisted of near-spherical particles with a narrow size distribution. Furthermore, the size assessed by TEM (about 100 nm) was smaller than that measured by DLS. This can be attributed to different conditions of the size measurements, i.e., application of dried and hydrated particles. More exactly, outer shell of the associates could collapse during the TEM experiment [47].

The associates were also studied by the UV–vis spectroscopy and no significant changes were observed in their spectra (Fig. 8). The macrocycle **3** showed the absorption bands at 258 and 280 nm corresponded to the transitions in the aromatic system of thiacalixarene. A slight hyperchromic effect and bathochromic shift by 2 nm were observed in the **3 + SDS** system. This confirms the formation of these stable particles detected earlier by DLS and TEM.

In the spectrum of paclitaxel, the absorption band was observed with the maximum at 245 nm that characterized the aromatic fragments of the compound. The absorption band of low intensity of the carbonyl group ($\lambda_{max} \geq$ 270–290 nm) was not visible probably because of the rise of the baseline when light was scattered by undissolved paclitaxel. The drug is implemented into the associate **[3 + SDS]** via either electrostatic or hydrophobic–hydrophobic interactions / Van der Waals interactions between the paclitaxel and the hydrophobic scaffold of macrocycle and hydrophobic tail of surfactant (Fig. 7). The stability of the **[3 + SDS] + Paclitaxel** system decreased as results from increased baseline caused by the coagulation of the paclitaxel particles. The rise in the baseline of the spectrum is related to the slight opalescence caused by incomplete solubilization of paclitaxel by mixed particles **[3 + SDS]** (Fig. 8).

3. Conclusion

Associates of the water–soluble thiacalix[4]arenes functionalized with phosphonate and guanidinium fragments in the *1,3–alternate* conformation with anionic and cationic surfactants have been obtained for the first time. As was established, the macrocycle **3** with positively

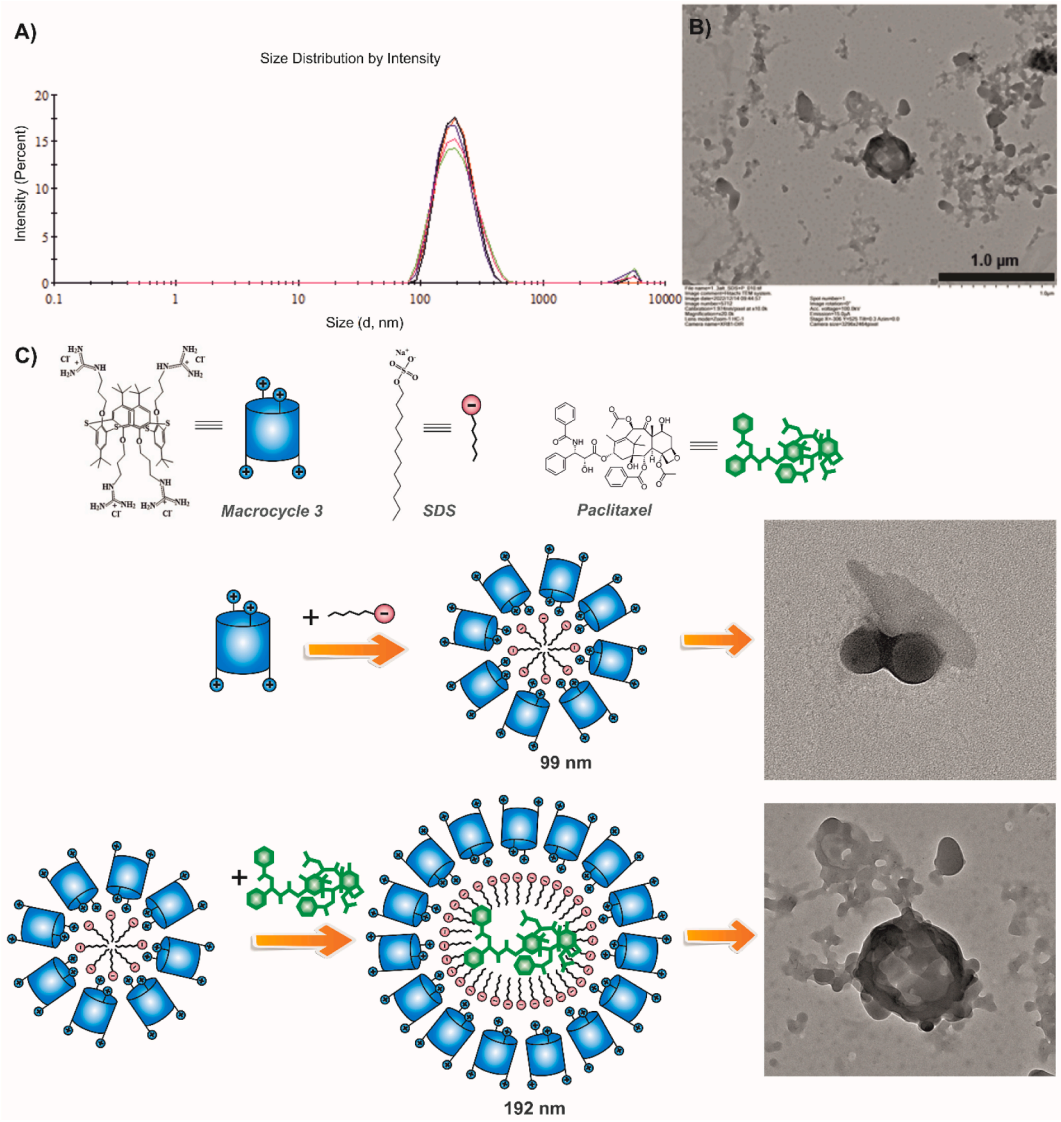

Fig. 7. (A) Size distribution, (B) TEM image of the particles formed by **[3 + SDS]** ($C = 1 \times 10^{-4}$ M) in the presence of paclitaxel and (C) the proposed scheme of paclitaxel solubilization by mixed particles **[3 + SDS]**.

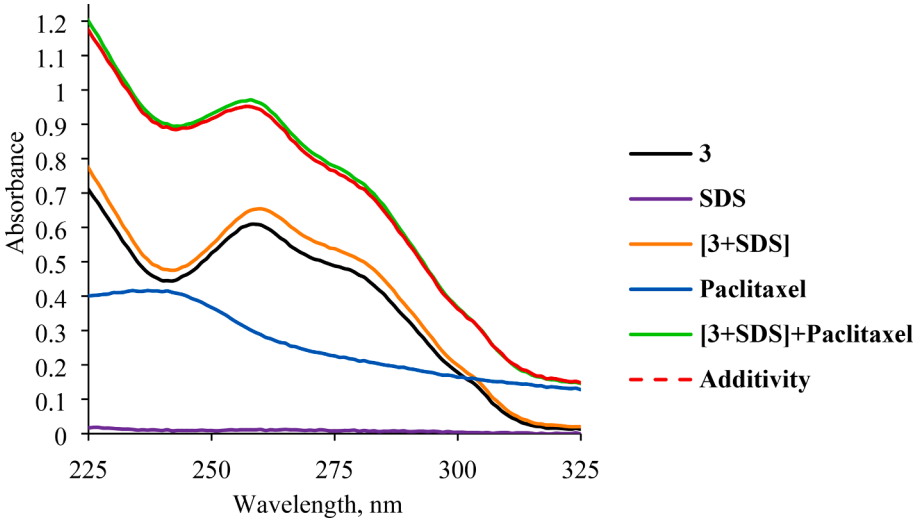

Fig. 8. Absorption spectra of the macrocycle **3** (5×10^{-5} M), **SDS** (5×10^{-5} M), their equimolar mixture with paclitaxel (5×10^{-5} M) and the ternary system **[3 + SDS] + Paclitaxel** in aqueous solution.

charged groups formed stable nanoparticles with **SDS** ($\zeta = 51 \pm 4$ mV) in the ratio of 1:1. Mixed micelles of the thiacalix[4]arene containing guanidinium fragments with **SDS** formed stable system with water–insoluble paclitaxel (d = 192 ± 7 nm, $\zeta = 33 \pm 4$ mV, PDI = 0.19 ± 0.04). The obtained interpolyelectrolyte complexes and mixed micelles based on thiacalix[4]arene derivatives and surfactants offer new opportunities for solubilizing water–insoluble drugs and creating new targeted drug delivery systems.

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CRediT authorship contribution statement

L.S. Yakimova: Data curation, Writing – original draft, Validation. **V.R. Sultanaev:** Investigation. **A.A. Vavilova:** Investigation, Data curation. **K.S. Shibaeva:** Data curation, Investigation. **I.I. Stoikov:** Conceptualization, Data curation, Writing – review & editing, Project administration, Funding acquisition.

Declaration of competing interest

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

Data availability

The authors do not have permission to share data.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.molliq.2023.123836.

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