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# Aggregation and Solubilization Properties of System Based on Glycine–Calix[4]resorcinol and Sodium Dodecyl Sulfate in Aqueous Medium

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*A complex of physicochemical methods showed that the water-insoluble glycine-based calix[4]resorcinol is solubilized by sodium dodecyl sulfate (SDS) micelles, thus not affecting the character of surfactant aggregation. pH-dependent solubilization of hydrophobic drug 2,2'-bibenzimidazole (BBI) in SDS micellar solutions in the presence of this macrocycle was shown. At acidic conditions the presence of solubilized calix[4]resorcinol in SDS micelles leads to a decrease in the joint solubilization of BBI in aqueous micellar solutions. In this case, the protonation of the nitrogen atom at the upper rim of calixarene favors its solubilization in the SDS micelles due to electrostatic interaction. This leaves little "room" in the hydrophobic part of the micelles to solubilize the drug. In the alkaline medium, the upper rim of calixarene is recharged to negative values due to the dissociation of hydroxyl and carboxyl groups. The electrostatic repulsion between SDS and calixarene molecules frees the hydrophobic part of the surfactant micelles from the macrocycle, which increases the solubilization capacity with respect to BBI. This result is of importance from the viewpoint of the delivery of lipophilic drugs.*

**Keywords**: Calixarene, surfactant, bibenzimidazole, drug, solubilization, aggregation.

# Агрегация и солюбилизационные свойства системы на основе глицинового каликс[4]резорцина и додецилсульфата натрия в водной среде

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*Комплексом физико-химических методов показано, что плохо растворимый в воде глициновый каликс[4]резорцин солюбизируется мицеллами додецилсульфата натрия (ДСН), не оказывая влияния на характер агрегации ПАВ. Установлена pH-зависимая солюбилизирующая способность мицеллярных растворов ДСН в присутствии данного макроцикла по отношению к гидрофобному лекарственному препарату 2,2'-бибензимидазолу (ББИ). В кислой среде присутствие солюбилизированного каликс[4]резорцина в мицеллах ДСН приводит к уменьшению совместной солюбилизации ББИ в водных мицеллярных растворах. В этом случае протонирование атома*

*азота на верхнем ободе каликсарена способствует его солюбилизации в мицеллах ДСН благодаря электростатическому взаимодействию, что в свою очередь оставляет мало места в гидрофобной части мицелл для солюбилизации лекарства. В щелочной среде верхний обод каликсарена приобретает отрицательный заряд из-за диссоциации гидроксильных и карбоксильных групп. Электростатическое отталкивание между ДСН и молекулами каликсарена освобождает гидрофобную часть мицелл ПАВ от макроцикла, что увеличивает способность к солюбилизации по отношению к ББИ. Этот результат имеет важное значение с точки зрения создания систем доставки липофильных лекарств.*

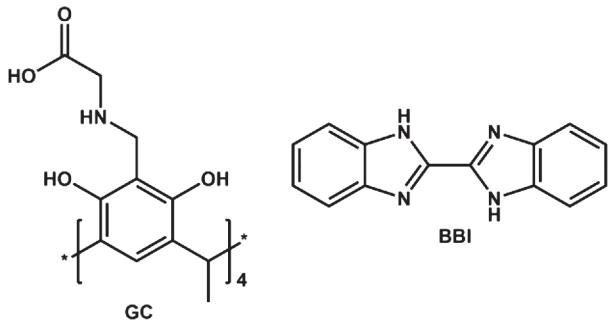
**Ключевые слова**: Каликсарен, ПАВ, бибензимидазол, лекарство, солюбилизация, агрегация.

## Introduction

The scientific and practical potential of the objects of supramolecular chemistry is great. Mixed compositions based on the building blocks of rigid (calixarenes, cyclodextrins) and soft (surfactant, polymers) structures invariably appear in the focus of scientific research, since they allow the development of multifunctional and smart materials, systems and structures to be promoted due to aggregation ability.[1-6] The formation of these mixed compositions can reduce the consumption of reagents[1,7] and connect the factors of morphology control of functional aggregates.[8-10]

A review of the literature shows that in recent years a significant number of calix[4]resorcinol derivatives have been synthesized, and their aggregation properties in the absence and in the presence of surfactants have been studied.[8-14] Despite the fact that surfactants in this case can play the role of a matrix facilitating the processes of self-aggregation, the mixed surfactant systems with calixarene containing biocompatible fragments are much less studied. Aggregation of surfactants in the presence of calixarenes containing amino acid groups has not been studied at all. Using amino acid groups in the calixarene structure causes the development of the applications as drug delivery agents,[15] detection of biomolecules[16] and metal ion absorbents.[17]

In order to create nanoscale supramolecular systems, the aggregation properties of calix[4]resorcinol functionalized with glycine groups at the upper rim (GC, Scheme 1) and its mixture with surfactant were investigated. Sodium dodecyl sulphate (SDS) served as a surfactant additive in mixed solutions. The p*H*-dependence behavior of amino acids concluded that a portion of amino acid groups of GC could be protonated in acidic conditions to give an ammonium ion and thus acquire a positive charge, thereby leading to the electrostatically driven mixed self-assembly with anionic surfactant. In this context, the main purpose of this work was to evaluate the effect of p*H* on the mixed aggregation of GC-SDS mixture and to demonstrate functional activity of this system as nanocontainers for water-insoluble antitumor drug 2,2'-bibenzimidazole (BBI).




**Scheme 1.** Molecular structures of GC and BBI.

## Experimental

Syntheses of C-methylcalix[4]resorcinol with glycine groups at the upper rim[13] and 2,2'-bibenzimidazole (BBI)[18,19] were described elsewhere. 1-Phenylazo-2-naphthol (Sudan I, Acros Organics) and SDS (99%, Acros Organics) were used as received. The water used for the preparation of all solutions was produced by a purification system (Millipore, Direct-Q 5).

The absorbance was measured on a Specord 250 Plus spectrophotometer (Analytic Jena, Germany) equipped with WinAspect software at 25±0.1 °C in a 1 cm path-length quartz cell. Absorbance for each sample was obtained by subtraction of the contribution of components to the summary spectrum. Reproducibility was checked for selected samples and no significant differences were observed.

Fluorescence emission spectra were done on a Cary Eclipse fluorescence spectrophotometer (USA). A quartz cell of 1 cm path length was used for all fluorescence measurements. Temperature of 25 °C was maintained. The excitation and emission slit widths for BBI fluorescence measurements were 2.5 nm and 5 nm, respectively.

Dynamic light scattering (DLS) studies were conducted at 25 °C using a Zetasizer Nano instrument (Malvern, UK) equipped with a 4 mW He-Ne laser operating at 633 nm. Correlation data were fitted using the method of cumulants to the logarithm of the correlation function, yielding the diffusion coefficient. Backscattered light was detected at 173°, and the intensity-average hydrodynamic diameter was calculated using the Stokes-Einstein equation. Zeta potential Nano-ZS (Malvern) with laser Doppler velocimetry and phase analysis light scattering was used for zeta potential measurement. The temperature of the scattering cell was controlled at 25 °C. All DLS scattering data were processed using Malvern Zetasizer Software.

## Results and Discussion

It is known that the solubility of GC in water is low (0.02 mM),[13] but a water-soluble form can be obtained by solubilization in surfactant micelles. The aggregation behaviour of SDS in an aqueous medium in the presence of solubilized GC was studied at the first stage by tensiometry (Figure 1a) and conductometry (Figure 1b) methods. Figure 1a shows the surface tension isotherm for the binary system consisting of SDS saturated with GC. The surface tension versus SDS concentration plot is very similar

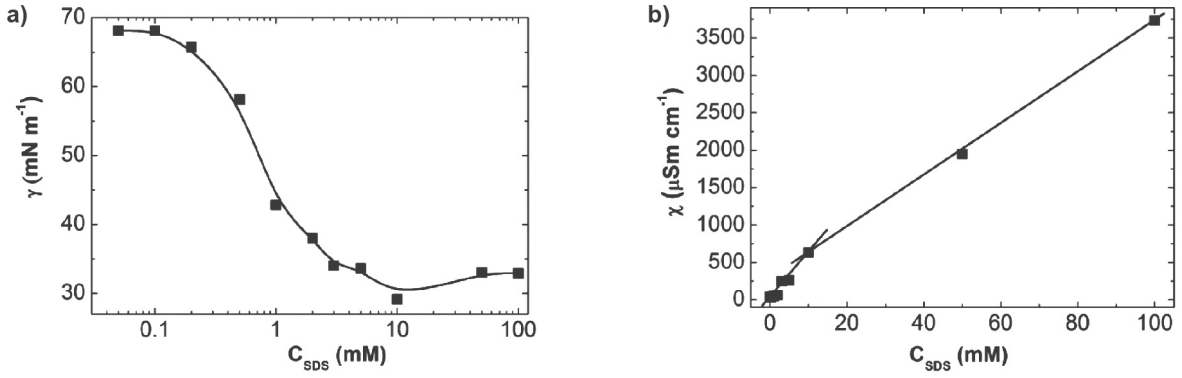



**Figure 1.** Concentration dependence of surface tension (a) and specific electrical conductivity (b) in aqueous solutions of SDS saturated with GC, 25°C.

to those for typical surfactants and demonstrates a breakpoint at the concentration of 8 mM. This value is almost identical with breakpoint observed in specific conductivity versus SDS concentration plot and coincides with critical micelle concentration (CMC) of the single SDS solution. According to DLS data, the mixed GC-SDS system exhibited monomodal particle distribution with mean value of hydrodynamic diameter of 2.5 nm (Figure 2), which is comparable with the size of individual SDS micelles. SDS micelles slightly increase in size in the presence of macrocycle as a result of their interaction. The solubilization of GC in the hydrophobic region of SDS micellar aggregates insignificantly affects the size of the latter. Hence, the presence of a solubilized macrocycle does not affect the value of the CMC of SDS.

The p*H*-dependent aggregation mechanism was studied for mixed GC-SDS system with 100 mM surfactant solution saturated with macrocycle. To determine the patterns of aggregation in this system, the solutions at p*H* 4.0 and 9.0 were investigated. In an acidic environment, mixed aggregates have a negative zeta potential (–58.0 mV) (Figure 3a) which is different from the zeta potential of an individual SDS micelles (–67.8 mV). This confirms the solubilization mode, when SDS micelles take place and their surface charge is compensated by positively charged GC molecules. In a weakly alkaline medium, a zeta potential shift towards –51.2 mV is observed (Figure 3b), which can be caused by an increase in the degree of binding of counterions and compensation of the negative charge by sodium ions. It is important to make it clear that above a p*H* of 4 there is virtually no change in the CMC for SDS.[20] Thus, the use of SDS constant concentration (100 mM) in solution saturated with a macrocycle provides comparable conditions to the CMC when the p*H* of the medium changes.

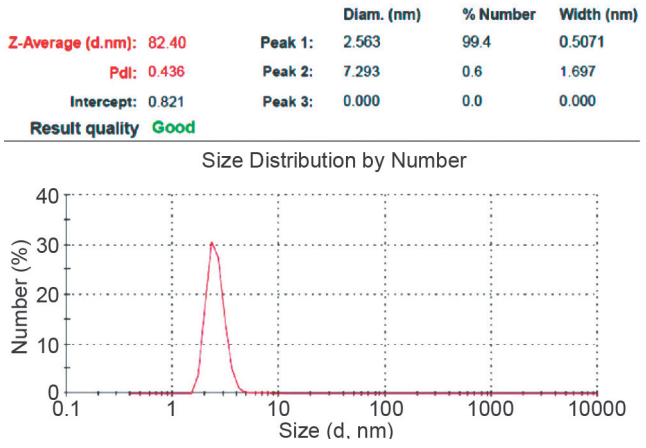



**Figure 2.** DLS results obtained for 100 mM SDS aqueous solution saturated with GC, 25 °C.

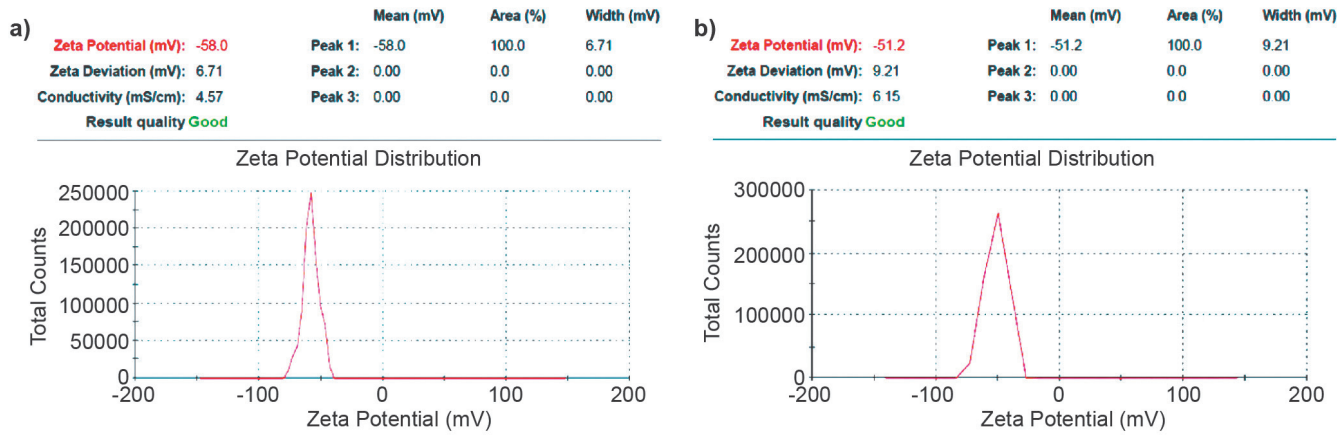



**Figure 3.** Zeta potential measurements of 100 mM SDS aqueous solution saturated with GC at p*H* 4 (a) and 9 (b), 25 °C.

Further, to elucidate the nature of aggregates formed, a highly hydrophobic probe Sudan I was used. Sudan I is solubilized by nonpolar interior of the micelles that is reflected by the absorbance value in the UV spectrum centered at 486 nm.[8] When comparing the results of the solubilization capacity toward Sudan I of the mixed SDS–GC system with the capacity of the individual SDS system, it was revealed that calixarene reduces the solubilization of Sudan I in SDS micellar structures (Figure 4), *i.e.* GC occupies the hydrophobic core of the surfactant micelles. The same was confirmed in the experiment with the antitumor drug 2,2'-bibenzimidazole (BBI). The UV spectrum of BBI solubilized with SDS micelles in an aqueous solution with a surfactant concentration of 20 mM is characterized by a maximum absorption band at 341 nm (Figure 5). Intensity of this band is reduced in the presence of GC, which confirms the same regularities of solubilization as in the case of the model Sudan I. Interestingly, absorption of BBI is further reduced when the medium is acidified. The experiments performed with BBI confirmed that morphology of mixed SDS–GC aggregates at p*H* 4 differs from that of aggregates formed at p*H* 9.

Also, the BBI molecule fluoresces at a wavelength of 392 nm upon excitation at 341 nm (Figure 6). It should be noted that in the presence of GC the fluorescence of BBI decreases, which correlates with the intensity of absorption and also indicates a lower solubility of BBI in the mixed GC-SDS system than in the individual SDS solution. A release of BBI from the interior of GC-SDS micelles was accompanied by a decrease in fluorescence emission upon acidification to p*H* 4. Taking into account a positive charge in an upper rim of GC due to a protonation of glycine groups, an electrostatic interaction with anionic groups of SDS can contribute to the solubilization of macrocycle in SDS micelles. This leads to the fact that there is little "room" in surfactant hydrophobic core for BBI solubilization.

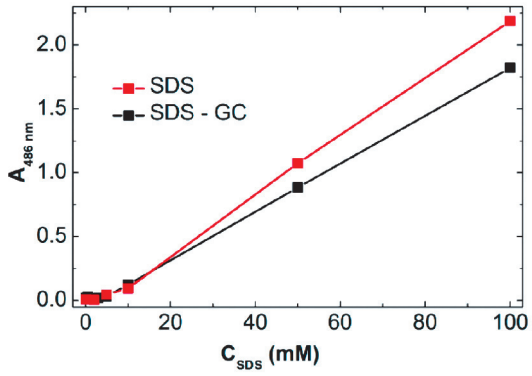



**Figure 4.** Concentration dependence of absorbance of Sudan I dye at 486 nm in aqueous solutions of SDS saturated with GC, optical path 0.1 cm, 25 °C.

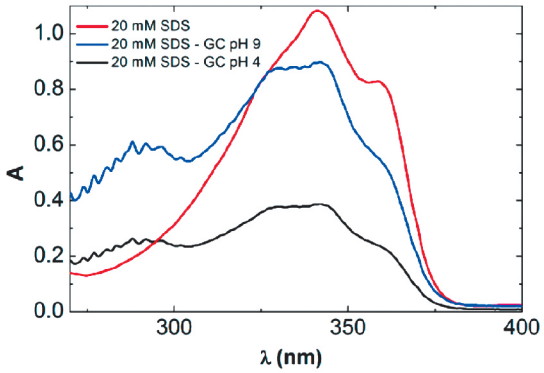



**Figure 5.** Absorption spectra of BBI in 20 mM SDS aqueous solution and 20 mM SDS solutions saturated with GC at p*H* 4 and p*H* 9, optical path 0.1 cm, 25 °C.

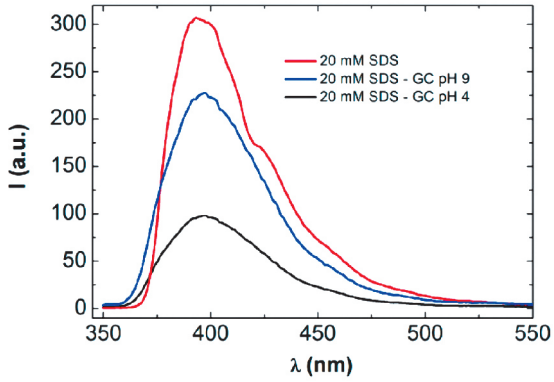
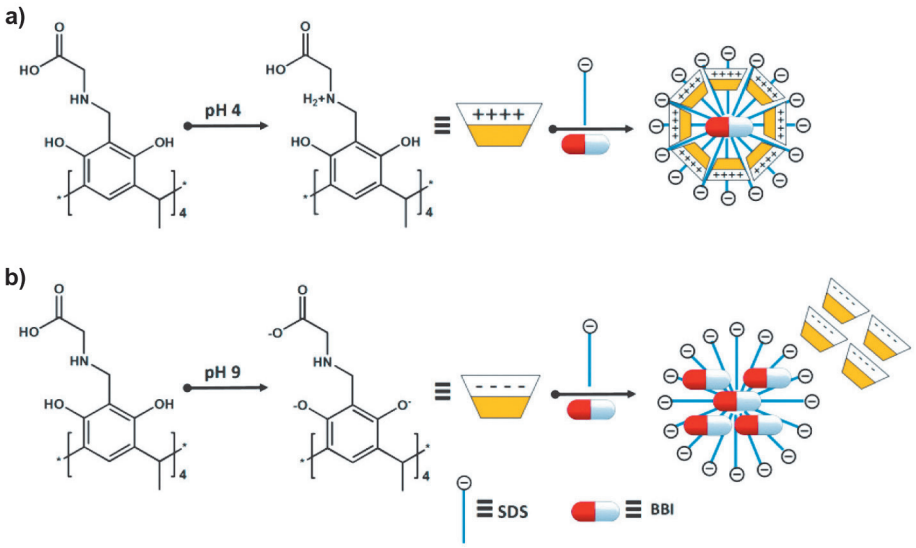



**Figure 6.** Fluorescence spectra of BBI in 20 mM SDS aqueous solution and 20 mM SDS solutions saturated with GC at p*H* 4 and p*H* 9, 25 °C.

Summarizing the results obtained, it can be assumed that the cause of the different solubilization capacity of SDS in the presence of GC is the different ionic form of calixarene macrocycle depending on p*H*. The protonation of the nitrogen atom in the acid medium on the upper rim of GC favors the solubilization of the macrocycle in the SDS micelles due to electrostatic interaction (Scheme 2a). At the same time, the joint solubilization of hydrophobic drugs is reduced due to the partial employment of the hydrophobic core of micelles by calixarene molecules. In the alkaline medium, the upper rim of calixarene is recharged: negative charges are present on the upper rim instead of a positive charge due to the dissociation of hydroxyl and carboxyl groups (Scheme 2b). The electrostatic repulsion between SDS and GC frees the hydrophobic part of the surfactant micelles from the macrocycle, which increases the solubilization capacity with respect to the hydrophobic drug. Therefore, the binding and release behavior of hydrophobic drugs in GC-SDS system can be governed by variation of the p*H*. This p*H*-dependent difference in solubilization may be used for design of nanocontainers with controllable binding capacity toward highly lipophilic drugs.

## Conclusions

A complex of physicochemical methods showed that the poorly soluble glycine-based calix[4]resorcinol is solubilized by SDS micelles, thus not affecting the character of SDS aggregation, *i.e.* does not change the critical micelle




**Scheme 2.** Schematic representations of solubilization of BBI in SDS micellar solutions in the presence of GC at p*H* 4 (a) and p*H* 9 (b).

concentration of this surfactant. p*H*-dependent solubilization of hydrophobic drug 2,2'-bibenzimidazole (BBI) in SDS micellar solutions in the presence of this macrocycle was shown. At acidic conditions the presence of solubilized calix[4]resorcinol in SDS micelles leads to a decrease in the joint solubilization of 2,2'-bibenzimidazole in aqueous micellar solutions. In this case, the protonation of the nitrogen atom at the upper rim of calixarene favors its solubilization in the SDS micelles due to electrostatic interaction. This leaves little "room" in the hydrophobic part of the micelles to solubilize the drug. In the alkaline medium, the upper rim of calixarene is recharged to negative values due to the dissociation of hydroxyl and carboxyl groups. The electrostatic repulsion between SDS and calixarene molecules frees the hydrophobic part of the surfactant micelles from the macrocycle, which increases the solubilization capacity with respect to the 2,2'-bibenzimidazole. This finding can be used for the design of nanocontainers with p*H*-dependent binding/release of lipophilic drugs.

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