Open Access **Baghdad Science Journal** **P-ISSN: 2078-8665**
Published Online First: April, 2023 **2023, 20(6): 2086-2105** **E-ISSN: 2411-7986**

DOI: https://dx.doi.org/10.21123/bsj.2023.7960

In vivo evaluation the efficiency of nitazoxanide with cationic Gemini surfactant on Cryptosporidiosis

Zeinab Ahmed*[1]** ***Eman M. Kandeel*[1]** ***Hagar F. Abdelmaksoud*[2] ***Tarek Aboushousha*[3]** ***Entsar E. Badr*[1]**

[1]Faculty of Science for Girls, University of Al-Azhar, Cairo, Egypt.
[2]Department of Parasitology, Theodor Bilharz Research Institute, Giza, Egypt.
[3]Department of Pathology, Theodor Bilharz Research Institute, Giza, Egypt.
*Corresponding author: Pearlhfn@yahoo.com
E-mail address: zenabahmed@azhar.edu.eg , dremankandeel@azhar.edu.eg , T.aboushousha@tbri.gov.eg , entsar@azhar.edu.eg

Received 15/10/2022, Revised 17/2/2023, Accepted 19/2/2023, Published Online First 20/4/2023, Published 01/12/2023

This work is licensed under a Creative Commons Attribution 4.0 International License.

Abstract:

Infection with cryptosporidiosis endangers the lives of many people with immunodeficiency, especially HIV patients. Nitazoxanide is one of the main therapeutic drugs used to treat cryptosporidiosis. However, it is poorly soluble in water, which restricts its usefulness and efficacy in immunocompromised patients. Surfactants have an amphiphilic character which indicates their ability to improve the water solubility of the hydrophobic drugs. Our research concerns the synthesis of new cationic Gemini surfactants that have the ability to improve the solubility of the drug Nanazoxide. So, we synthesized cationic Gemini surfactants. N^1,N^1,N^3,N^3-tetramethyl-N^1,N^3-bis(2-octadecanamidoethyl)propane-1,3-diaminium bromide (CGSPS18) and 2,2`-(ethane-1,2-diylbis(oxy))bis(N-(2-octadecanamidoethyl)-N,N-dimethyl-2-oxoethane-1-aminium) dichloride (CGSES18) and the detection of their chemical composition by spectroscopic methods, as well as studying the properties of their surfaces and their toxicity. Furthermore, the efficacy of nitazoxanide in infected mice was studied in conjunction with three different doses of surfactants. To assess the effect of nitazoxanide and surfactants, the infection was parasitologically counted before and after treatment, and the intestinal, liver, and lung tissues were also examined histopathologically. In this study, it was found that the combination of the drug nitazoxanide with surfactants, especially the compound (CGSPS18) at a concentration of 25% increased the efficacy and resulted in a percentage reduction of 90.8%. Histopathological examination revealed that the group treated with the drug nitazoxanide in combination with CGSPS18 showed the best results exhibiting an almost normal villous pattern. This study demonstrated an increase in the effectiveness of nitazoxanide when combined with surfactants, and this suggests a promising future for the use of surfactants as an adjunct to enhance the effectiveness of nitazoxanide for the treatment of cryptosporidiosis in immunocompromised patients, particularly HIV patients.

Keywords: Cationic Gemini Surfactant, *Cryptosporidium*, HIV patients, Immunocompromised, Nitazoxanide.

Introduction:

Cryptosporidium spp. is one of the most common intestinal parasites. It was first detected in mice in 1907 but was not identified as a major cause of diarrheal disease in humans until 1976, whether in healthy children or immunocompromised adults. The infection can be life-threatening and has been detected in more than 70 countries. The pathogen can be transmitted by many routes, including water and food, but is the most common cause of waterborne disease. It is estimated to have a high prevalence in children under five years of age, low-income people, people with gastrointestinal symptoms, the elderly, and people with immunodeficiency, especially HIV patients [1–3]. *Cryptosporidium parvum* (*C. parvum*) is a coccidian protozoan that causes cryptosporidiosis. It is immune to all chlorination levels, and it is a pathogen that lives only inside cells. The parasite's development is mainly limited to the intestine; in immunocompromised hosts, the biliary system,

pancreas, and lungs may also be affected [4,5]. There is also evidence that cryptosporidiosis is associated with the occurrence of cancer [6].

To date, there is no fully effective therapy for *Cryptosporidium* spp. treatment options are minimal, despite the overall burden of cryptosporidiosis. Nitazoxanide is the only drug approved by the U.S. Food and Drug Administration, and it is not effective in patients with immunodeficiency and not approved in infants younger than 1 year [7]. It acts by inhibiting pyruvate ferredoxin oxidoreductase (PFOR), thereby impeding anaerobic energy transfer reactions [8]. As nitazoxanide is used to treat cryptosporidiosis in children, its efficacy is limited in malnourished people and compromised immune systems, this highlights the significance of research towards medication development for the treatment of cryptosporidiosis. Nitazoxanide, a high-dose water-insoluble antiprotozoal drug, was developed with the aim of modulating gastro-retentive dosage forms using super porous hydrogel composites, which resulted in improved stability, drug content, and drug release. Furthermore, loading of chitosan nanoparticles with nitazoxanide increased their efficacy in both infected immunocompetent and immunosuppressed mice, resulting in a decrease in the shedding of *Cryptosporidium* oocyst and an improvement in the histopathological changes caused by the infection in the mice's liver, intestine, and lungs [9,10].

Surfactants were used as one of the components to prepare oil-in-water nanoemulsion, which improves the solubility of nitazoxanide and medical bioavailability, increases the therapeutic effect of the drug, reduces the dosage of the drug and increases the activity of resistance to parasites, worms, bacteria, etc., and is used to treat diseases caused by protozoa in humans and livestock, besides, it is safe to use and easy to prepare [11]. In another study, the dissolution enhancement of nitazoxanide was studied and compared with its two derivatives nitazoxanide-glutaric acid and nitazoxanide-succinic acid in the presence of two types of cellulosic polymers [12].

Surfactants have an amphiphilic character, containing both hydrophobic and hydrophilic portions. Often, aggregate at interfaces and are associated in solutions (water or oil phases) to attempt to isolate their different portions from the phase interaction. There are clearly many areas where applied chemistry can help in the future development of drug encapsulation by surfactants [13]. in an early study, two types of surfactants were used as antiprotozoal agents and showed antiprotozoal properties in sheep rumen [14]. Various drug delivery mechanisms and drug targeting have been studied with the aim of reducing drug degradation and losses, preventing adverse effects, and improving drug bioavailability [15]. One of the most classical techniques to improve the water solubility of hydrophobic drugs is solubilization in surfactant micelles, which is still employed in the pharmaceutical industry. [15–17].

Cryptosporidium spp. poses a threat to the lives of many people around the world. Therefore, we focused our attention on the most common drug for its treatment, namely nitazoxanide, but it is poorly soluble in water, which affects its efficacy. Our study aimed to prepare a cationic Gemini surfactant with high efficiency and minimal toxicity. A comparison of the efficacy of nitazoxanide on cryptosporidiosis alone, and in combination with surfactants at different concentrations was performed in *C.parvum* infected mice after induction of immunosuppression. Parasitological and histopathological examinations were performed to evaluate the effect of the drugs and surfactants.

Experimental:

Synthesis of cationic Gemini surfactant

Synthesis of (CGSES18)

It was prepared according to the approach described in Bin-Hudayb *et al.* [18].

(CGSPS18) was synthesized in two steps as follows

preparation of N-(2-(dimethylamino)ethyl) octadecanamide (DAEO)

At 35-40ºC, add dropwise octadecanoyl chloride 2.51 g, 0.0083 mol to a mixture of N,N-dimethyl ethylenediamine 2.42 g, 0.0275mol in 15ml dry ether. The reaction was agitated for 2 hours, and at the end of this period, the solvent had been evaporated under reduced pressure then filtered off as a precipitate and recrystallized with pure ethanol after washing in water. The final product was vacuum-dried [19].

preparation of (CGSPS18)

N-(2-(dimethylamino) ethyl) octadecanamide 7.09 g, 0.02 mol and 1,3-dibromopropane (PS) 2.02 g, 0.01 mol were placed in ethyl acetate (as solvent) in a 100 ml quick-fit flask equipped with a condenser. The flask was magnetic stirred under reflux for 24 hours. Once the solvent has been removed by evaporation under reduced pressure, the final product was dried under vacuum after recrystallization with petroleum ether see schema in Fig. 1 [20].

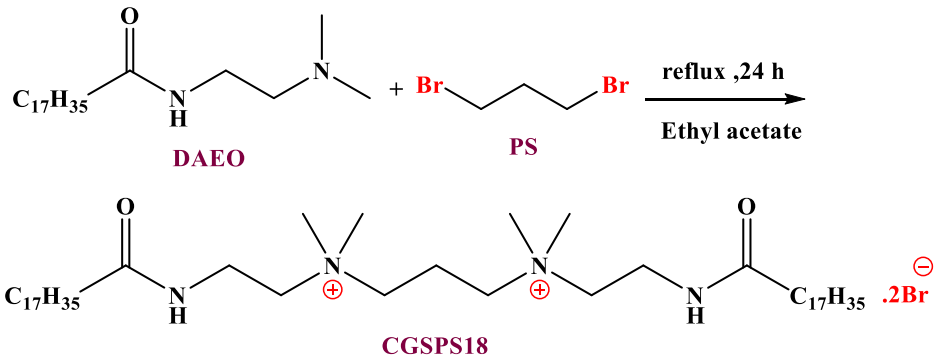


Figure 1. Synthesis of CGSPS18

Preparation of oocyst

The molecular laboratory of Theodor Bilharz Research Institute provided the *Cryptosporidium parvum* oocysts. By utilizing the appropriate primers in a nested PCR reaction, this strain's molecular identity was established from the start of the cycle, and the PCR result was subsequently sequenced [21]. Samples were first washed by 10 ml of N-saline centrifuged for 10 minutes at 3000 rpm, the supernatant was removed, and this process was done twice. The sediment was then re-suspended with Shether's solution and allowed to stand for 10 minutes. Next, collection of the supernatant, and it was saline-washed twice. The remaining oocysts in the deposit were counted using a hemocytometer to calculate the dose needed for infection. The dosage of infection was 10^3 *C. parvum* oocysts [22].

Animals

Animal source and handling

Fifty-four white albino female mice of the CDI strain were healthy, lab-bred, and between 6 and 8 weeks old, weighing between 25 and 30 grams (all obtained from Theodor Bilharz Research Institute animal house). The experiment was carried out in a plastic cage with clean Sawdust for bedding and a decent ventilation system at the biological section of Theodor Bilharz Research Institute. Mice were housed under standard environmental factors (12/12 h light/dark cycle), with a temperature of 24°C and a relative humidity of 50%. They were fed with a commercial diet and water and protected from direct sunlight to maintain optimum sanitation. All mice received Praziquantel 600mg at a single dose of 40 mg/kg and were allowed to rest for 7 days before the start of the experiment to ensure that the mice were free from the parasitic infection [23].

Groups of Animals

This study was carried out on fifty-four healthy, (lab-bred) white albino female mice, which were divided into nine groups, with six immunocompromised mice in each group. Mice in groups II to XV were infected with *C. parvum* oocysts at a dose of 10^3 oocysts/0.2ml/mouse orally via a nasogastric feeding tube.

Group. I: non-infected and non-treated (negative control).

Group. II: infected and non-treated (positive control).

Group. III: infected and treated with nitazoxanide in a dose of 200 mg/kg daily for 7 successive days after infection [10].

Group. IV: infected and treated with CGSES18 in a concentration of 25% at a half dose of 100 mg\ml [24] with nitazoxanide half the dose from it for 3 times\week for 7 successive days post infection.

Group. V: infected and treated with CGSES18 in a concentration of 50% at a half dose of 100 mg\ml [24] with nitazoxanide half the dose from it for 3 times\week for 7 successive days post infection.

Group. VI: infected and treated with CGSES18, in a concentration of 100% at a half dose of 100 mg\ml [24] with nitazoxanide half the dose from it for 3 times\week for 7 successive days post infection.

Group. VII: infected and treated with CGSPS18 in a concentration of 25% at a half dose of 100 mg\ml [24] with nitazoxanide half the dose from it 3 times\week for 7 successive days post infection.

Group. VIII: infected and treated with CGSPS18 in a concentration of 50% at a half dose of 100 mg\ml [24] with nitazoxanide half the dose from it for 3 times\week for 7 successive days post infection.

Group. IX: infected and treated with CGSPS18 in a concentration of 100% at a half dose of 100 mg\ml [24] with nitazoxanide half the dose from it for 3 times\week for 7 successive days post infection.

Doses were computed based on the table of (Barnes & Paget) [25].

Immunosuppression

Dexamethasone sodium phosphate (Dexazone), 0.25 ug/g/day, was supplied through nasogastric feeding tube to suppress the immune system. This dosage was given every day for two weeks before receiving an oral injection with *Cryptosporidium* spp. oocysts. All mice groups received a weekly maintenance dosage of dexazone throughout experiment [26,27].

Infection

Using a nasogastric feeding tube, mice were orally infected with *C. parvum* oocysts at a dosage of 10^3 oocysts/0.2 ml/mouse[22]. After conducting a pilot investigation, it was found that 10^2 oocysts/0.2 ml/mouse was insufficient to produce infection whereas 10^4 oocysts/0.2 ml/mouse resulted in the death of the mice.

Drugs and therapeutic doses

Medications were given orally, via a nasogastric feeding tube.

- Nitazoxanide: was given in a dose of 200 mg/kg daily for 7 successive days after infection [10].
- Surfactant CGSES18 & CGSPS18: was given in a dose of 100 mg\ml 3 times\week for 7 successive days post infection [24].

Experimental evaluation of treatment

Parasitological examination

Stool samples were collected from each group 7 days after infection. The therapeutic impact of the medications was then assessed using stool samples obtained 7 days after therapy. After being stained with the modified Ziehl-Neelsen (MZN) stain, samples were examined for parasites[28]. We used a microscope to count the *C.parvum* oocysts in each sample. The percentage (%) reductions in oocysts were calculated using the following Eq.1:

$$\%R = 100(C - E)/C. \quad 1$$

%R: % reductions, C: control group and E: experimental groups of mice [29].

Histopathological examination

All mice were scarificed 10 days after treatment. The ileocecal region, lungs, and liver were located and fixed in 10% buffered formalin solution and processed into paraffin blocks. Sections 4-5 um thick were cut using a rotatory microtome (Leica, Germany) in the pathology department of TBRI. Routine staining was done using Hematoxylin and Eosin(H&E) for demonstrating the different pathological changes using a light microscope (Zeiss, Axio) [30], with an attached digital camera Micr5 for taking microphotographs for interesting findings.

Statistical analysis

Data were analyzed using paired sample t-test and independent sample t-test by using predictive analytic software IBM SPSS Statistics (Version 25), *P* value is considered significant if < 0.05 [31].

Results and discussion:

Chemistry

Two types of cationic Gemini surfactants were prepared. The first contains an ester spacer (CGSES18) and the second a propane spacer (CGSPS18). They were prepared by acylation of N,N-dimethyl ethylenediamine with octadecanoyl chloride in dry ether, leading to the production of (DAEO) as intermediates. These were agitated separately with ethane-1,2-diylbis(2-chloroethanoate) or 1,3-dibromopropane, respectively, to synthesize the required surfactants after 24 h.

Construction explanation: Fourier-transform infrared (FTIR), nuclear magnetic resonance (NMR), and mass spectroscopy (MS) were used to determine the structures of all the cationic Gemini surfactants (CGSES18) and (CGSPS18) prepared. The (FTIR) spectra of the (DAEO) substance indicated a strong absorption band at 1624–1644 cm^{-1}, typical of carbonyl amide, and a strong signal in the region of 3002–3056 cm^{-1}, which is characteristic of NH stretching. There was a significant peak at 1761 cm^{-1} corresponding to the absorption of carbonyl of the ester group for CGSES18, in addition to the distinctive intermediate peaks (DAEO) as shown in Table 1, Figs. 2,3,4.

Table 1. FT-IR data of cationic Gemini surfactant.

Cationic Gemini Surfactants	FT-IR Data (cm^{-1})				
	(ν N–H)	ν C=O Amide	ν C=O Ester	ν C-H Asym.	ν C-H Sym.
DAEO	3002	1644	----------	2697	2624
CGSES18	3334	1624	1761	2914	2836
CGSPS18	3056	1634	----------	2767	2706

Baghdad Science Journal
2023, 20(6): 2086-2105
P-ISSN: 2078-8665
E-ISSN: 2411-7986

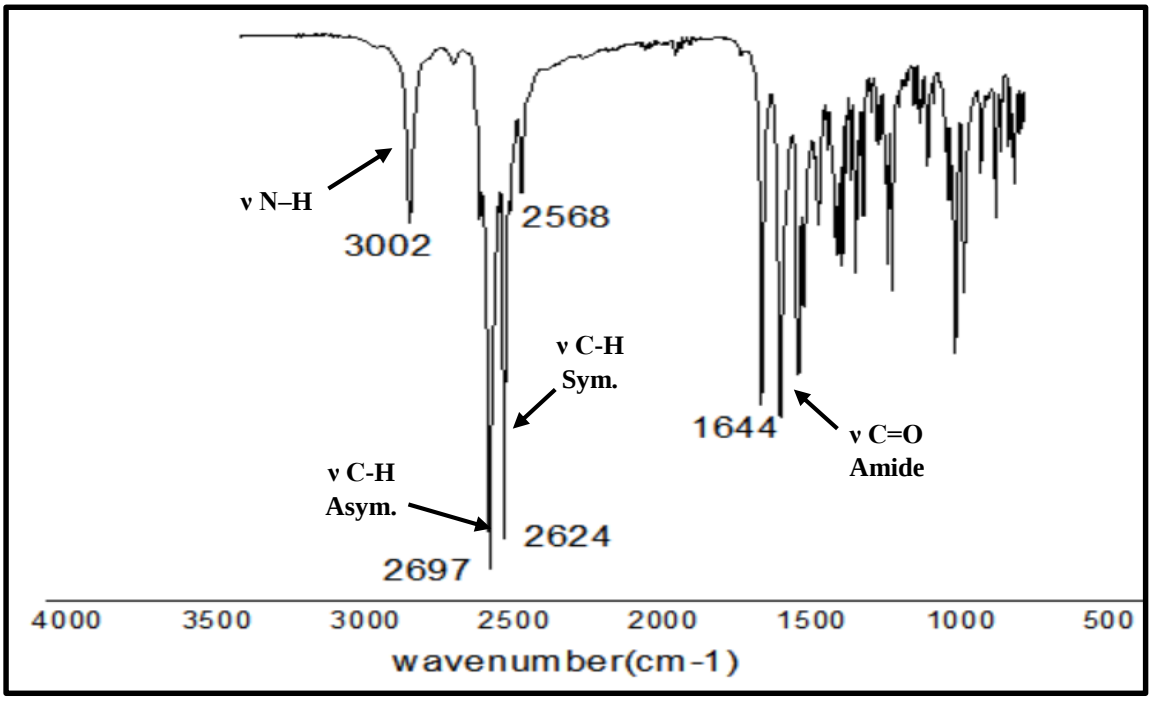


Figure 2. FT-IR Spectra for DAEO

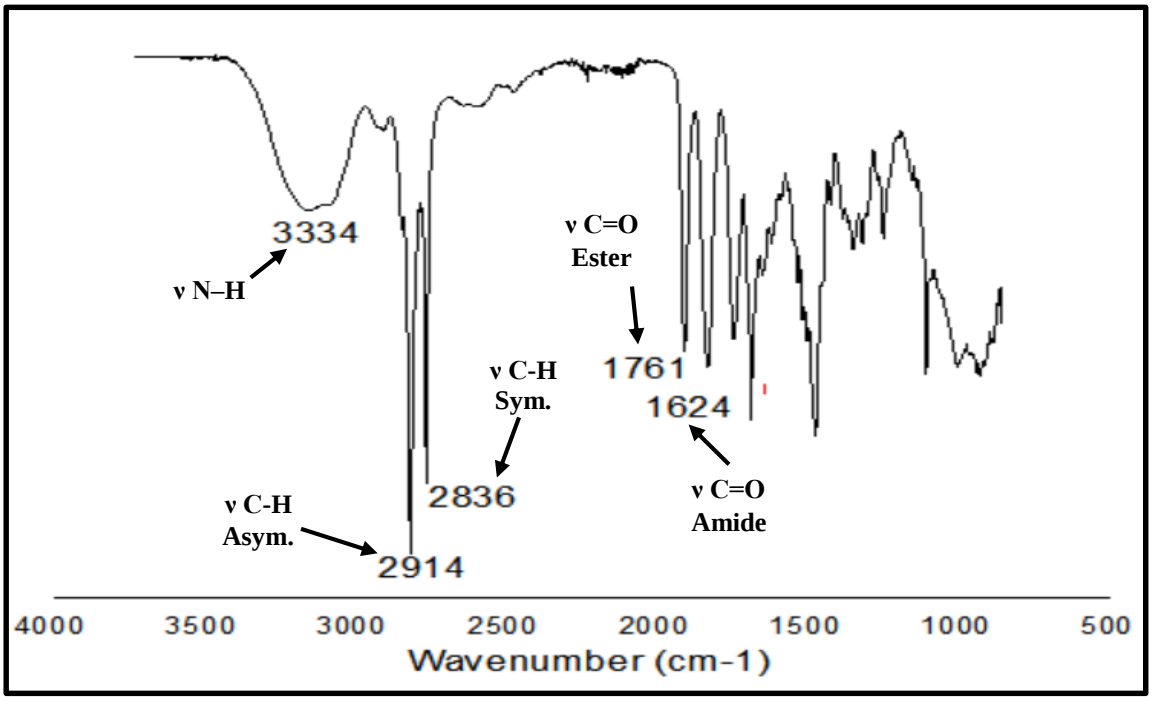


Figure 3. FT-IR spectrum for CGSES18

Baghdad Science Journal
2023, 20(6): 2086-2105
P-ISSN: 2078-8665
E-ISSN: 2411-7986

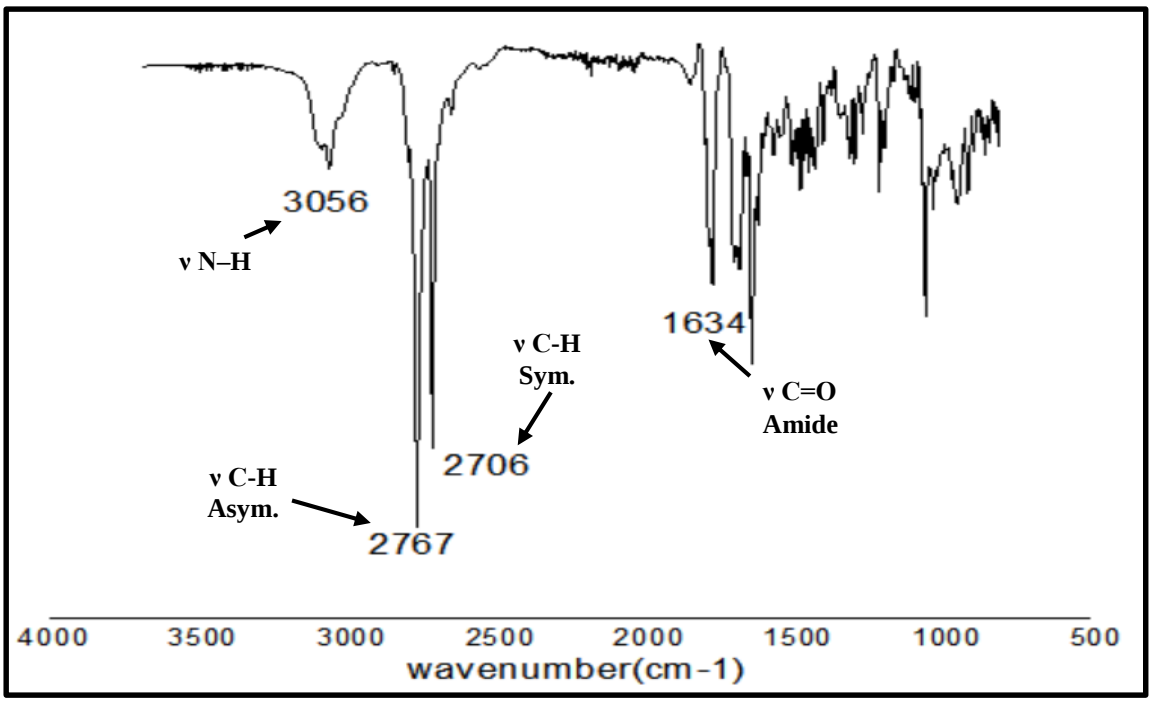


Figure 4. FT-IR spectrum for CGSPS18

According to the ^{1}H-NMR data for DAEO, CGSES18, and CGSPS18 in Table 2, Figs. 5,6,7. The chemical shifts for protons (CH_3CH_2) of the lipophilic tail of the cationic Gemini surfactants CGSES18 and CGSPS18 were 0.88, 0.89, and 1.24, 1.26 ppm, respectively. At δ (2.21 – 2.88) ppm, there was a methylene proton peak before the carbonyl (C=O) group, that belongs to the fatty chain. At δ 3.55, 2.68 ppm a singlet of ($C\underline{H}_3)_2$ protons directly linked to the positively charged quaternary nitrogen [$N^+(C\underline{H}_3)_2$] was detected. The methylene protons between the two nitrogen atoms, i.e., N- $C\underline{H}_2$ - $C\underline{H}_2$-N^+, are responsible for the peaks at 3.94, 2.93, and 3.68, 2.90 ppm. At δ 4.51 and 2.32 ppm for CGSES18 and CGSPS18, respectively, typical resonance protons of the methylene group (-$C\underline{H}_2$-) were found to be directly linked to the quaternary nitrogen that has a positive charge, which is a part of the spacer. CGSES18 has a methylene proton peak next to the ester group, which is part of the spacer, at δ 5.14 ppm, and CGSPS18 has a methylene proton signal next to N^+-CH_2 -$C\underline{H}_2$ at 3.35 ppm. In contrast to CGSPS18, which had a singlet at 7.72 ppm, the resonance of a proton bonded to nitrogen in CGSES18 is displaced to the up field and is impacted by the ester group of the spacer, that was a singlet at 8.48 ppm.

Table 2. ^{1}H NMR reading describing the type of proton in cationic Gemini surfactant.

Cationic Gemini Surfactants	Proton NMR (δ in ppm, 850 MHz, $CDCl_3$)									
	Tail				Amide		Amido-Amine		Spacer	
	CH_3 (a)	CH_2 (b)	CH_2 (c)	CH_2 (d)	NH (e)	CH_2 (f)	CH_2 (g)	CH_3 (h)	CH_2 (i)	CH_2 (j)
DAEO	0.86	1.24	1.59	2.16	6.12	3.31	2.41	2.24	------	------
CGSES18	0.88	1.25	1.57	2.21	8.48	3.94	3.68	3.55	4.51	5.14
CGSPS18	0.89	1.26	1.61	2.88	7.72	2.93	2.90	2.68	2.32	3.35

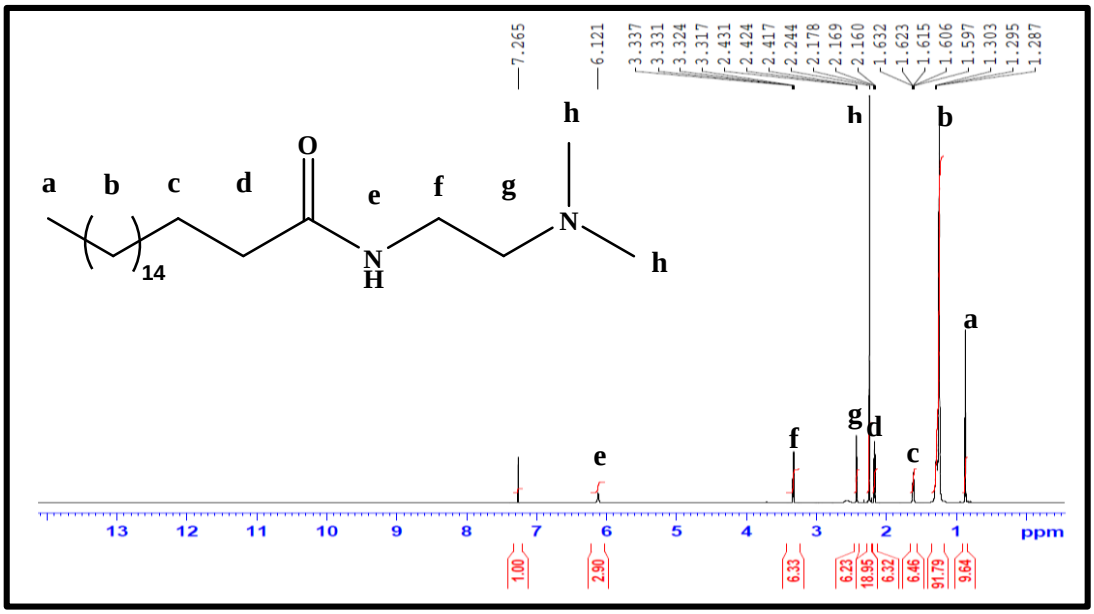


Figure 5. ^{1}H-NMR Spectra for DAEO

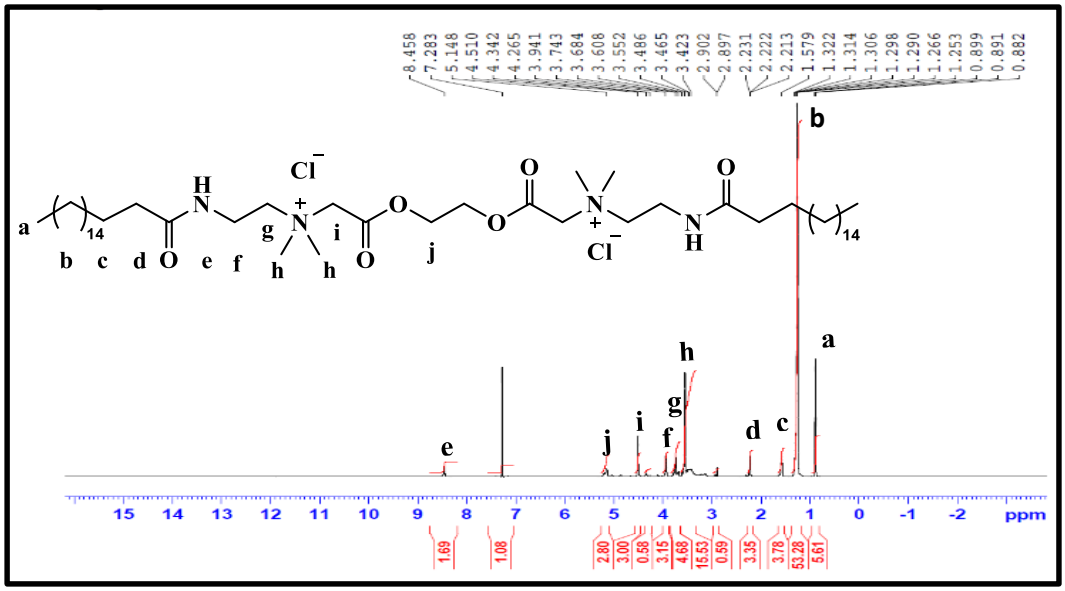


Figure 6. ^{1}H-NMR Spectra for CGSES18

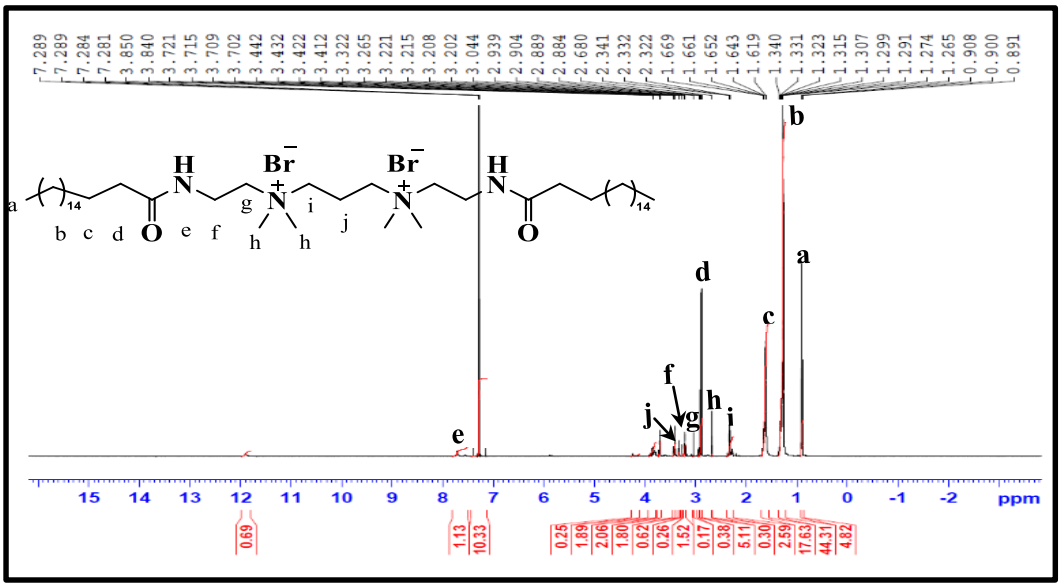


Figure 7. ^{1}H-NMR Spectra for CGSPS18

^{13}C NMR for DAEO (δ in ppm, $CDCl_3$ solvent at 213 MHz):14.16, 22.72, 25.82, 29.35, 29.39, 29.72, 29.67, 31.95, 36.56, 36.79, 45.09, 57.92, 175. As shown in Fig. 8 and for CGSES18 ^{13}C NMR (δ in ppm, $CDCl_3$ solvent at 213 MHz):14.17, 22.73, 25, 60, 29.35, 29.42, 29.54, 29.73, 29.84, 29, 31.97, 34.07, 52.80, 59.57, 61.35, 63.24, 67.95, 164.71, 175.10. as shown in Fig. 9.

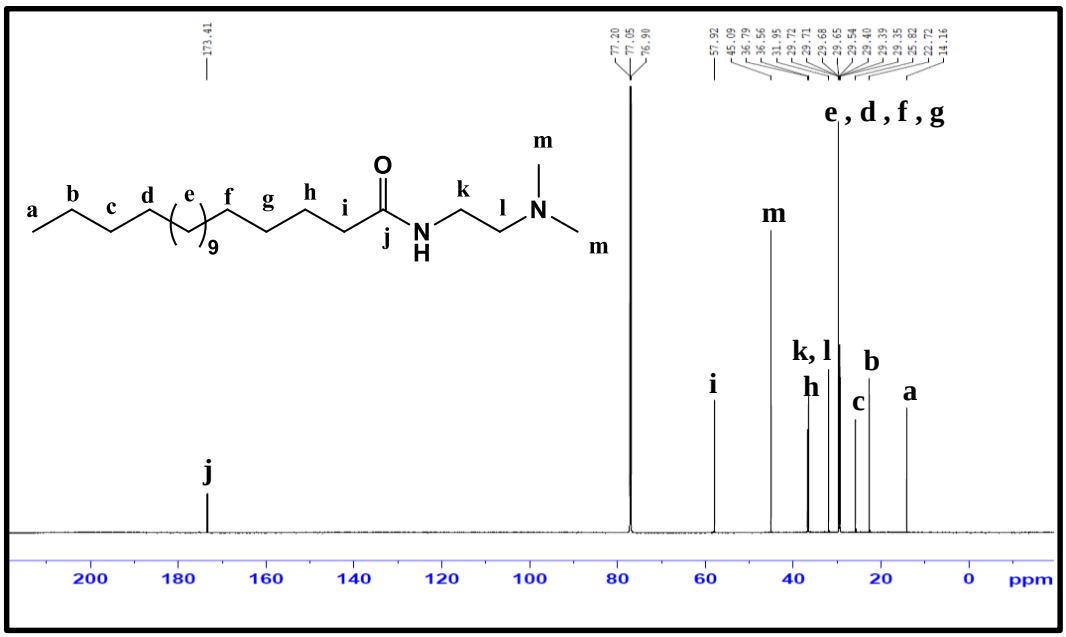


Figure 8. ^{13}C-NMR Spectra for DAEO

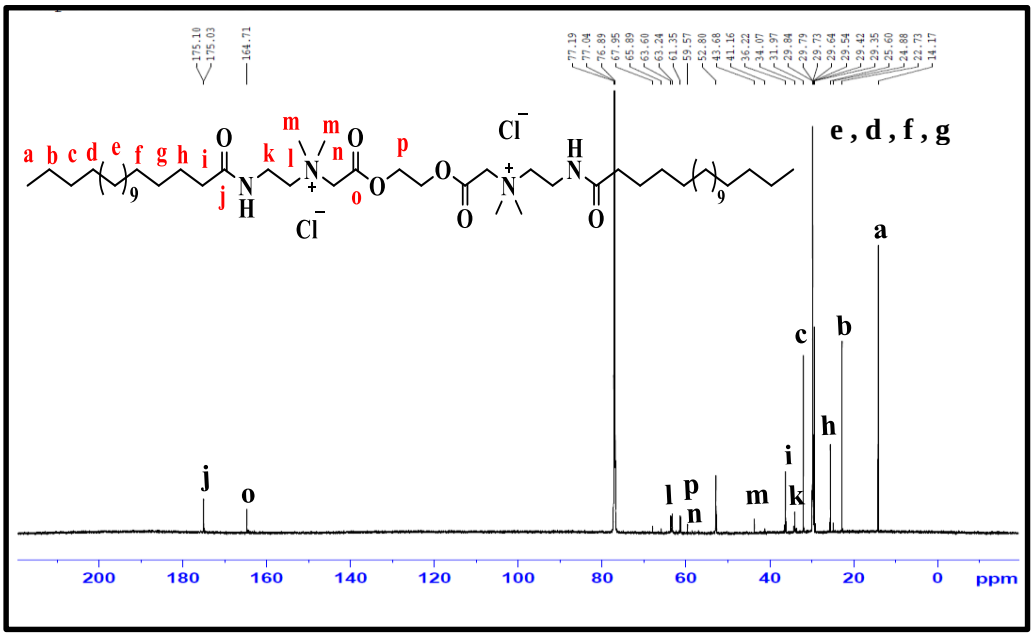
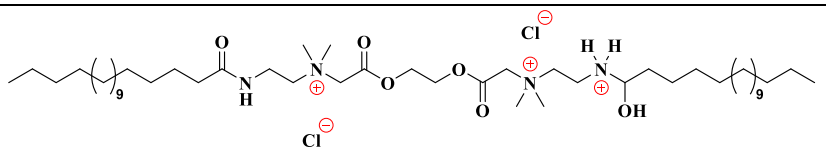
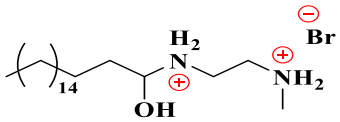


Figure 9. ^{13}C-NMR Spectra for CGSES18

In mass spectroscopy, the chemical structure of all evaluated contemporary cationic Gemini surfactants was validated using mass spectral data. The calculated m/z values for the molecular weight of cationic Gemini surfactant 924.27 and 911.14 were in perfect agreement with the practical values for (924, 911) for CGSES18 and CGSPS18, as shown in Table 3, Figs. 10,11 .

Table 3. GC-MS data of CGSES18 and CGSPS18.

Cationic Gemini Surfactants	M. Wt. Calculated/found	Proposed Structure (Base Peak)
CGSES18	924.27/923.26	 (913.10)
CGSPS18	911.14/911.71	 (424.27)

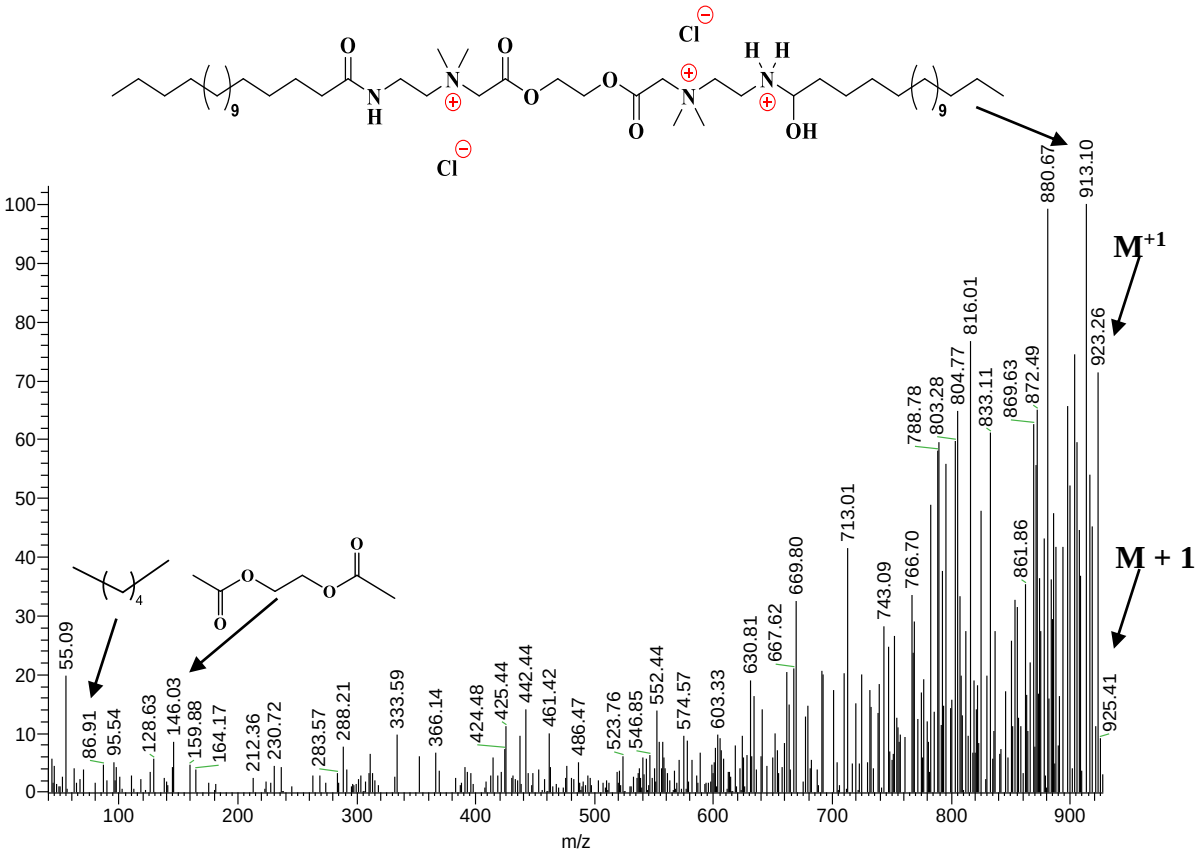


Figure 10. Mass spectrum for CGSES18

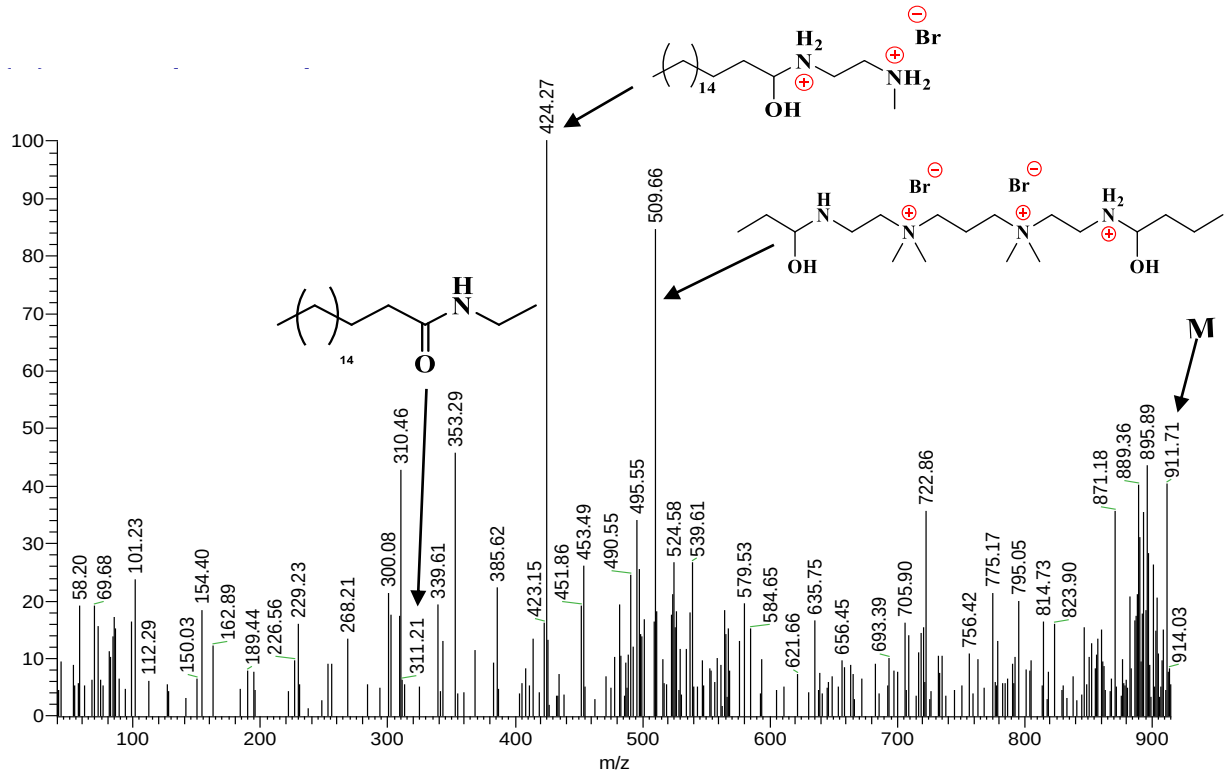


Figure 11. Mass spectrum for CGSPS18

Surface tension measurements

Critical Micelle Concentration (C_{cmc}) and Effectiveness (π_{cmc})

The C_{cmc} value was calculated by plotting the surface tension (γ) against (LnC) as shown in Fig. 12. It was determined using the breakpoints of the graphs presented in Table 4. It was observed that with increasing surfactant concentration up to C_{cmc}, a significant linear decrease in surface tension was observed for CGSES18 and CGSPS18, with a greater decrease in surface tension for CGSES18 than CGSPS18. Thus, the effectiveness (π_{cmc}) at C_{cmc} is higher for CGSES18 than for CGSPS18 and is estimated using the following Eq.2 [32–34].

$$\pi_{cmc} = \gamma_o - \gamma_{cmc} \quad (2)$$

Where γ_o is the surface tension of pure water and γ_{cmc} are the surface tension at C_{cmc}.

Table 4. Surface tension parameters of the synthesized cationic Gemini surfactants in double-distilled water in 25°C.

Surfactant Name	γ_{cmc}	C_{CMC} x 10^3	π_{cmc}	Γ_{max} x 10^{11}	A_{min}
	(mN m^{-1})	(mol L^{-1})	(mN m^{-1})	(mol cm^{-2})	(A^{o2} $molecule^{-1}$)
CGSES18	24	0.921	48.3	7.332	226.45
CGSPS18	31	2.02	41.3	5.602	296.4

C_{cmc} the critical micelle concentration, γ_{cmc} surface tension at C_{cmc}, Γ_{max} maximum surface excess concentration, π_{cmc} effectiveness, A_{min} minimum surface area per molecule at the air/solution interface.

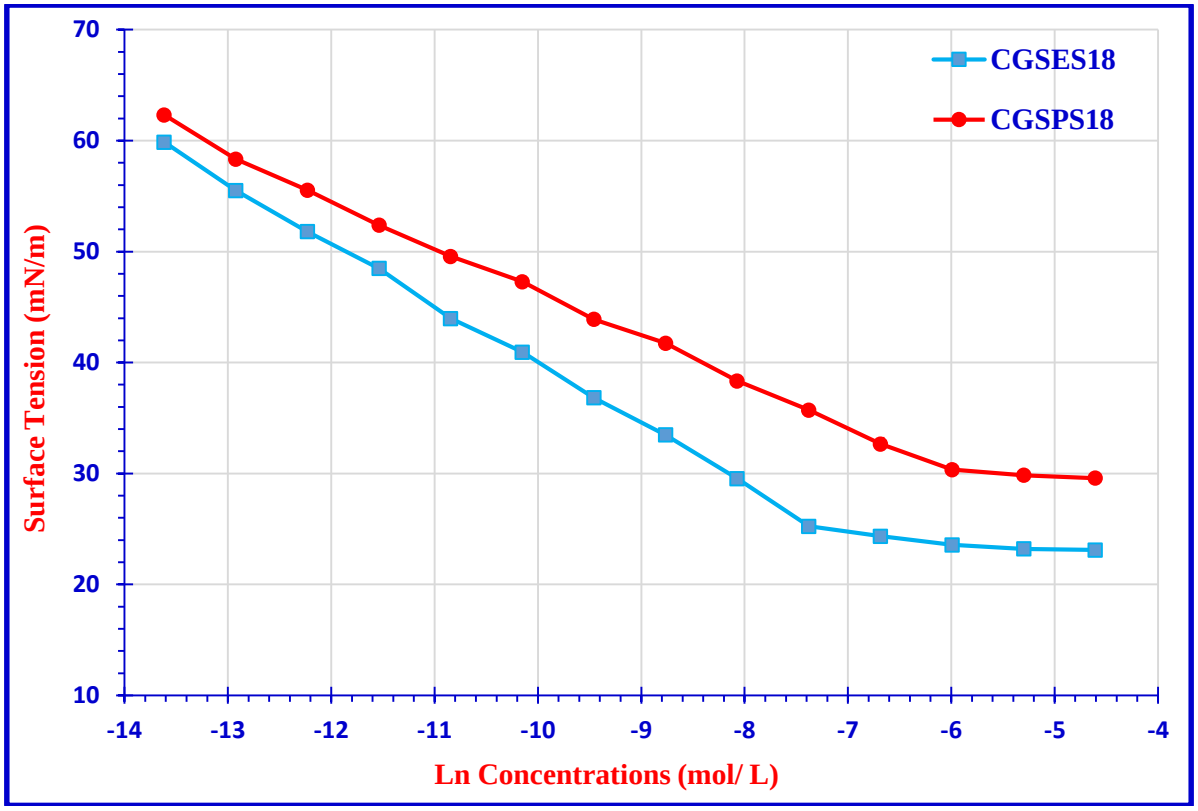


Figure 12. Variation of the surface tension of the synthesized cationic Gemini surfactant vs. Ln(concentration) in double-distilled water at 25°C

Surface Excess (Γ_{max}) and Minimum Surface Area (A_{min})

The efficiency of adsorption at the interface is called the maximum surface excess concentration Γ_{max}. And the minimum surface area occupied by any molecule adsorbed at the air–solution interface is called A_{min}. Γ_{max} and A_{min} were calculated using the Gibbs adsorption Eqs. 3 and 4, which is as follows [35–37]:

$$\Gamma_{max} = - [1/n\ RT]\ [d\gamma\ /d\ LnC\ ]_T \qquad 3$$

$$A_{min} = 10^{16} / [\Gamma_{max}\ N_A] \qquad 4$$

Plotting γ versus lnC, the slope refers to dγ/dlnC. In comparison to the bulk solution, the surfactant concentration near the surface was always greater because the surfactants in the solution prefer the air/water interface over the bulk solution [38].

Table 4 displays the calculated values for Γ_{max} and A_{min}. The Γ_{max} and A_{min} values indicate whether the amphiphiles are densely or loosely packed at the surface [39].

We found that the various spacers have little effect on the calculated Amin values. There is a significant difference in the calculated A_{min} values between CGSPS 18and CGSES18, due to CGSPS18 with propane spacer attached to the quaternary ammonium group has a greater Amin value than that of similar ester functionalized Gemini surfactants. The polar character of the spacer in CGSES18, which has a stronger affinity for the aqueous phase, is to blame for this behavior, that makes CGSPS18 stretch at the air–water interface. As shown in Fig. 13 [40,41].

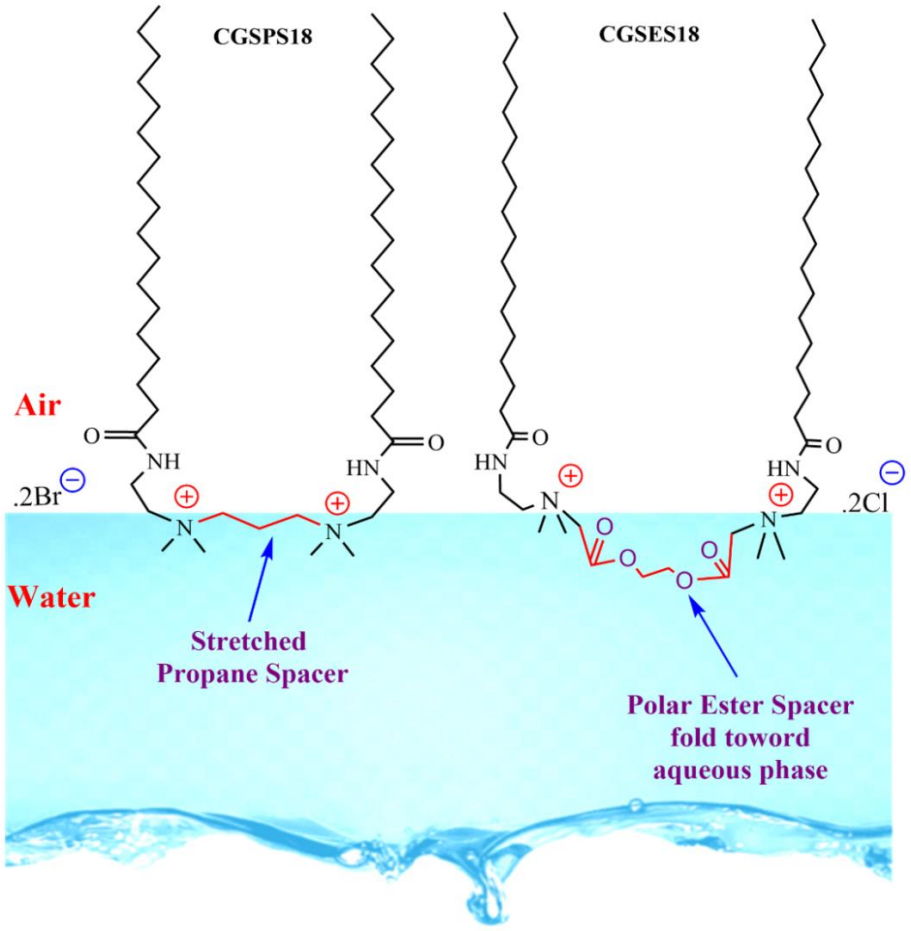


Figure 13. Schematic representation of the cationic Gemini surfactants at the air–water interface

Surfactant molecules dissolve in water at a concentration greater than the critical micelle concentration (CMC), forming micelles, while the hydrophilic heads remain on the micelle's outer surface to increase water interaction, the hydrophobic tails congregate at its center to reduce water contact [42].The aqueous phase penetrates the micelle beyond the hydrophilic head groups, with the hydration sphere consisting of the first few methylene groups close to the head as the hydration sphere [15,43]. As a result, micelles can improve the solubility of sparingly soluble compounds in water, which is particularly useful in the pharmaceutical industry. Solubilization is the reversible interaction between a substance's micelles and a surfactant to cause a material to dissolve spontaneously in water [42]. Since the drug nitazoxanide has an intermediate solubility, it should be placed in the middle of the micelle, e.g., between the CGSES18 and CGSPS18 micelles' hydrophilic head groups [44]. The solubilization of this drug could be mostly owing to adsorption at the micelle-water interface rather than incorporation into the micelle interior [45].

Evaluation of cytotoxicity against WI-38 cell line

Inhibitory activity against normal human lung fibroblast cells was observed for CGSES18 under these experimental conditions with CC50 = 21.33 ± 1.08 µg/ml as shown in Fig. 14, and for CGSPS18 CC50 = 39.16±2.37 µg/ml, as shown in Fig. 15. The complete evaluation of cytotoxicity against the WI-38 cell line was doing according to Mosmann *et al.* and Abd-El-Aziz *et al.*[46,47].

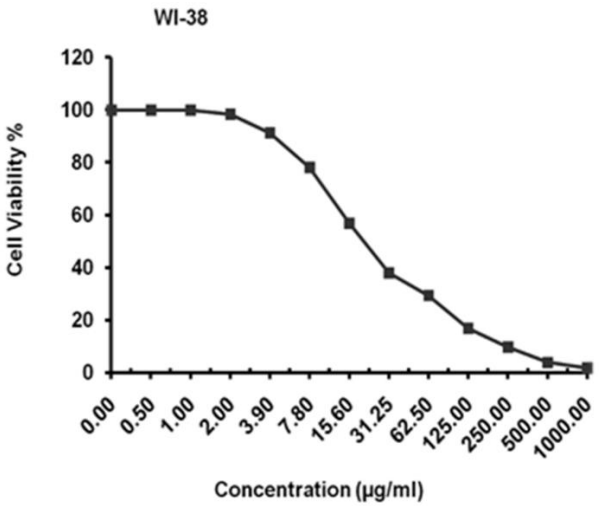


Figure 14. Evaluation of cytotoxicity for CGSES18

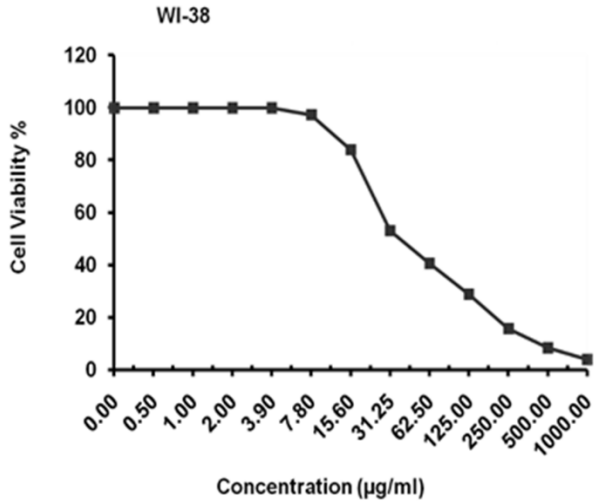


Figure 15. Evaluation of cytotoxicity for CGSPS18

Parasitological examination

Cryptosporidiosis is a parasite that causes diarrheal diseases that affect the majority of people worldwide [48]. In both developed and developing countries, it is considered a serious problem, especially for immunocompromised patients [49], particularly children under five years of age, the elderly, and people with immunosuppression, especially HIV/AIDS patients [2,50]. In addition to the numerous side effects of available drugs, and the weakness of their efficacy. Our study addressed the drug nitazoxanide as one of the most important drugs for the treatment of *Cryptosporidium* spp. [7], and its weak efficacy is due to its poor solubility in water, resulting in low bioavailability and delivery problems [9,51]. Therefore, cationic Gemini surfactants with a solubilizing effect have been developed for poorly soluble drugs [52]. The effects of CGSES18 and CGSPS18 at different concentrations on *Cryptosporidium* spp. in combination with nitazoxanide were investigated. The results were compared with the untreated positive control group and the group taking nitazoxanide alone. The parasitological studies revealed that there were highly significant statistical differences between the untreated positive control and all groups, regardless of whether they had taken nitazoxanide alone or in combination with surfactant, they also show highly significant statistical differences between the group that took nitazoxanide alone and the groups that took nitazoxanide with surfactant at all different concentrations (*P*-value < 0.05), as shown in Tables 5,6.

Table 5. The results of parasitological examination of different groups compare with positive Control by using Paired Samples Test.

Pair		Mean	Std. Deviation	t	*P* value
	Nanazoxide (After Drug)	185.83	3.545		
I	positive Control (before Drug)	1562.83	3.488	-637.427	
	CGSES18 25% + Nanazoxide (After Drug)	214.00	3.033		
II	positive Control (before Drug)	1562.83	3.488	-708.171	
III	CGSES18 50% + Nanazoxide (After Drug)	265.50	3.271		
	positive Control (before Drug)	1562.83	3.488	-530.618	
IV	CGSES18 100% + Nanazoxide (After Drug)	250.17	3.545	-693.981	< 0.05*
	positive Control (before Drug)	1562.83	3.488		
V	CGSPS18 25% + Nanazoxide (After Drug)	143.50	3.450	-587.101	
	positive Control (before Drug)	1562.83	3.488		
VI	CGSPS18 50% + Nanazoxide (After Drug)	170.17	2.994	-929.593	
	positive Control (before Drug)	1562.83	3.488		
VII	CGSPS18 100% + Nanazoxide (After Drug)	160.00	3.688	-581.105	
	positive Control (before Drug)	1562.83	3.488		

p value calculated using paired sample T-test
**P* value is considered significant if < 0.05

Table 6. The Result of the t-Test (Independent sample) between Group treated with Nanazoxide only and Nanazoxide + Surfactants.

	Groups	N	t	*P* value
Number of *Cryptosporidium* (After Drug)	Nanazoxide	6	-14.788	< 0.05*
	CGSES18 25% + Nanazoxide	6		
	Nanazoxide	6	-40.456	
	CGSES18 50% + Nanazoxide	6		
	Nanazoxide	6	-31.433	
	CGSES18 100% + Nanazoxide	6		
	Nanazoxide	6	20.964	
	CGSPS18 25% + Nanazoxide	6		
	Nanazoxide	6	8.270	
	CGSPS18 50% + Nanazoxide	6		
	Nanazoxide	6	12.370	
	CGSPS18 100% + Nanazoxide	6		

*Equal variances assumed

It was observed that the lowest number of oocyst excretion 143.50± 3.450 with a percentage reduction of 90.8% was observed in group VII treated with CGSPS18 at 25% concentration in combination with nitazoxanide. Followed by group IX with a percentage reduction of 89.8% treated with (CGSPS18) at a concentration of 100% in combination with nitazoxanide. Followed by group VIII with a percentage reduction of 89.1% treated with (CGSPS18) at a concentration of 50% in combination with nitazoxanide, and then group III with a percentage reduction of 88.1% treated with nitazoxanide as shown in Fig. 16. The parasitological study showed that the combination of surfactants with nitazoxanide reduced the excretion of oocysts, especially in CGSPS18. This is due to the ability of cationic Gemini surfactants to solubility enhancement [53]. Our results went in agreement with *Gaggero et al.* [54] who use surfactants to enhance in vitro dissolution of poorly soluble drugs like praziquantel which use for the treatment of schistosomiasis. Also shown with Kolenyak-Santos *et al*. [55].

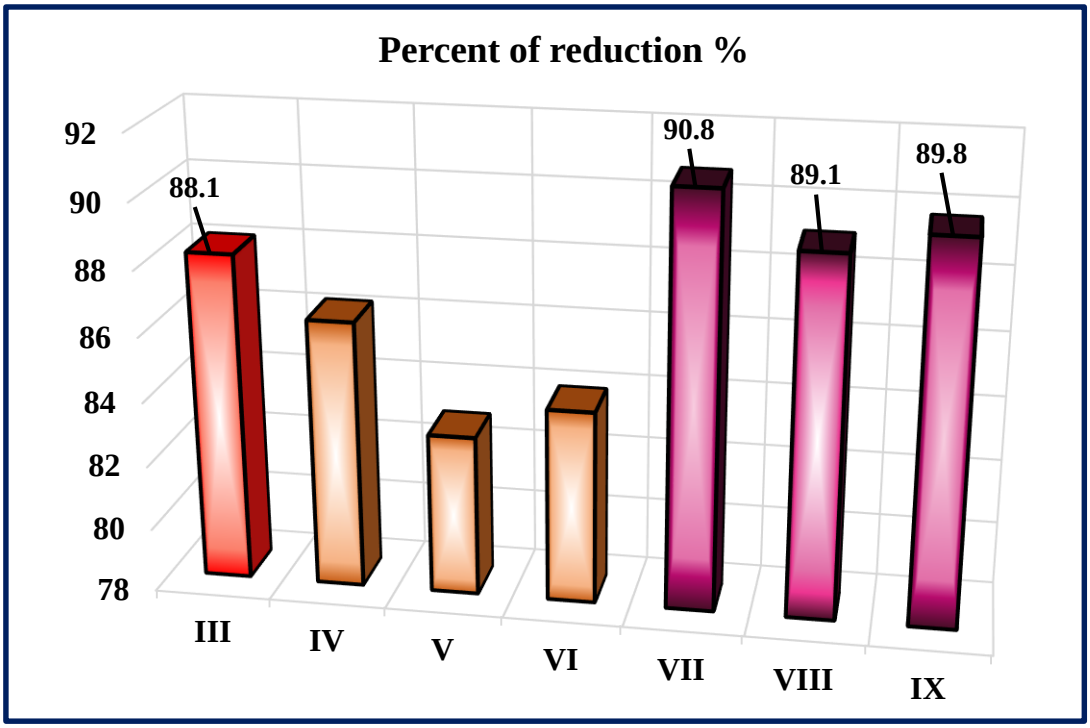


Figure 16. show percent of reduction *Cryptosporidium* Oocysts shedding

Histopathology assessment

Our work was the first to evaluate the combined therapeutic effect of cationic Gemini surfactant with nitazoxanide on cryptosporidiosis.

Examination of intestine

Histopathological examination of the ileocecal region of the mice in the different groups showed various degrees of pathological and neoplastic

lesions among them. These findings were in accordance with Abdelhamed *et al*.[56], and Fahmy *et al*.[57] who found that mice with infection from *Cryptosporidium* may cause dysplastic changes in the intestinal tract. Sadek *et al*.[58] reported that the pathological changes following the effect of nitazoxanide alone on intestinal tissue after infection with Cryptosporidiosis were moderate, our result show that groups VII, VIII, and IX treated with the nitazoxanide drug in combination with CGSPS18 showed the greatest efficacy, exhibiting an almost normal villous pattern like negative control mice at all drug concentrations. The groups treated with the nitazoxanide drug in combination with CGSES18, showed mild pathological changes in the form of mild villous changes, mild inflammatory cell infiltration and decreased mucin secretion at all concentrations compared to the positive control group. Group VI treated with CGSES18 at a concentration of 100% in combination with nitazoxanide showed nearly normal villous pattern like the negative control, except for the decreased mucous secreting pattern as shown in Fig. 17 – d. These results are in agreement with El-Wakil *et al*. who demonstrate the efficacy of combined regimen nitazoxanide in treating cryptosporidiosis, where normal mucosa, goblet cells, and a return to the normal villous pattern without any inflammatory cells were observed [59]. This increase in efficacy of nitazoxanide is due to combining it with cationic Gemini surfactant, that according to Fatma *et al*. [53], has the ability to enhance solubility. Group III treated with nitazoxanide alone showed revealed conventional histopathological features with normal villous architecture, absent or mild inflammation and normal mucin secreting pattern as shown in Fig. 17 - c.

Examination of liver sections

Histopathological examination of liver sections from different groups was concerned mainly with changes in hepatic architecture, hepatocytic changes, liver fibrosis, and inflammatory changes. Additionally, dysplastic liver changes following *C. parvum* infection has been observed in SCID mice [60]. The histopathological findings were mildly variable between different groups. Hepatocytic cloudy swellings and hydropic changes were minimal in negative and positive control mice as shown in Fig. 17- e,f respectively. It was most apparent in group IX treated with the nitazoxanide drug in combination with CGSPS18 at a concentration of 100% as shown in Fig. 17- g and mild in all other groups as in group VI treated with the nitazoxanide drug in combination with CGSES18 at a concentration of 100% as shown in Fig. 17-h. These findings are in line with those of Metawae *et al*. combined treatment with nitazoxanide demonstrated improvement of pathological changes in liver sections [61].

Examination of lung sections

Histopathological examination of lung sections from the different groups was concerned mainly with changes in the pulmonary vasculature and alveolar patterns as well as inflammatory changes. The histopathological findings were mildly variable between different groups like in negative group (I), positive group (II), a group treated with CGSES18 at a concentration of 50% in combination with nitazoxanide (V) and group treated with CGSPS18 at a concentration of 50% in combination with nitazoxanide (VIII) as shown in Fig. 17- i, j, k, l respectively. These findings were in agreement with Moawad *et al*.[10].

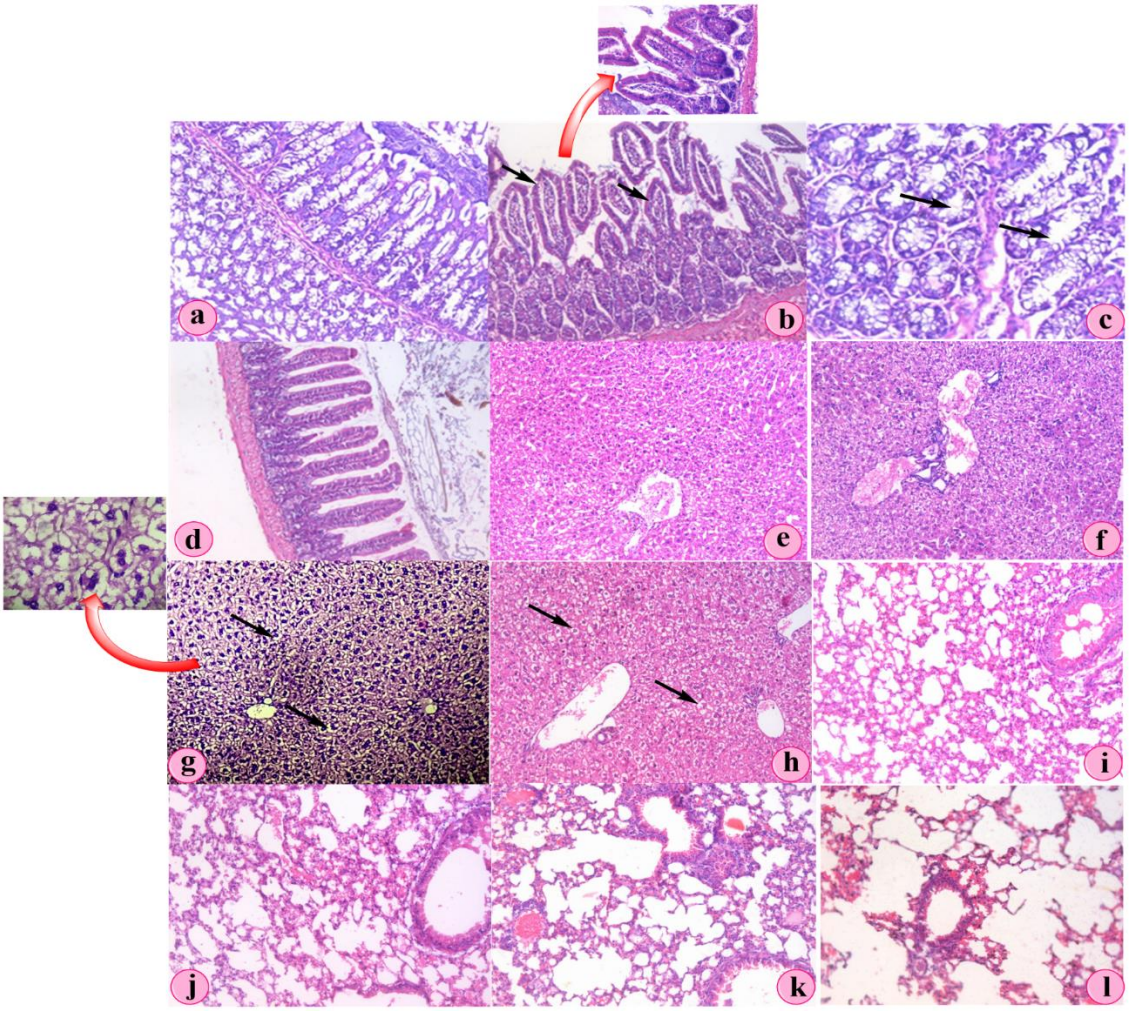


Figure 17. Sections of the intestinal from (a to d), hepatic tissues from (e to h) and lung section from (i to l) in different cases; (17-a): Negative control show normal villous architecture, absence or mild inflammation and normal mucin secreting pattern (H&E stain, X200); (17-b):is Positive Control Histopathological examination of sections from the intestine revealed villous broadening with decrease villous height to crypt length ratio. There was dense infiltration by mononuclear inflammatory cells within the villous core, degeneration of the villous tip-regions and increase mucin production (H&E stain, X200& X400); (17-c): showing group treated with nitazoxanide (H&E stain, X400); (17- d): showing no evidence of histopathological changes except for mild mucous secreting cell reduction (H&E stain, X200); (17- e): negative control sections showing unremarkable pathological changes (H&E stain, X200); (17-f): positive control mice showing unremarkable pathological changes (H&E, X200); (17-g): group treated with the nitazoxanide drug in combination with CGSPS18 at a concentration of 100% showing marked hydropic changes (H&E stain, X200 & X400); (17-h): group treated with the nitazoxanide drug in combination with CGSES18 at a concentration of 100% showing mild hydropic degeneration of hepatocytes (H&E stain, X200); (17-I,j): negative and positive control mice showing unremarkable pathological changes (H&E stain, X200)

Conclusion:

Two types of cationic Gemini surfactants were utilized in this study, one with an ester spacer (CGSES18) and the other with a propane spacer (CGSPS18). The efficacy of the drug nitazoxanide in this investigation was enhanced when it was combined with surfactants, particularly with the surfactant CGSPS18 at a concentration of 25%, which gave a percentage reduction of 90.8%. Histopathological examination showed that the group treated with the drug nitazoxanide in combination with CGSPS18 had the greatest efficacy and showed an almost normal villous pattern at all drug concentrations. This paves the way for the use of surfactants in the treatment of cryptosporidiosis with nitazoxanide to increase efficacy, as surfactants increase water solubility.

Acknowledgment:

This paper is based upon work supported by Science, Technology & Innovation Funding Authority (STDF) under grant number (44571). Deep thanks and gratitude to Prof. Dr. M. S. Taher (Chemistry Department, Faculty of Science, Al-Azhar University, Girls, Nasr City, Cairo, Egypt) for his interest, and useful discussions.

Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for republication attached with the manuscript.
- The author has signed an animal welfare statement.
- Ethical Clearance: The Ethical Committee at Theodor Bilharz Research institution (TBRI) approved the project and an ethical approval was obtained under ID: PT717.

Authors' contributions statement:

This work was carried out in collaboration between all authors. E.M.K, E. E. B and H. F. A-E; methodology. E.M.K, E.E.B. and Z. A; formal analysis and software. H. F. A-E and Z. Ahmed; Parasitological examination and data curation. T.A.; Histopathological examination. E.E.B, H.F.A-E and Z.A.; Writing original draft preparation and writing review and editing. E.M.K, E.E.B and H.F.A-E; supervision. All authors read and approved the final manuscript.

References:

1. Hunter PR, Nichols G. Epidemiology and clinical features of *Cryptosporidium* infection in immunocompromised patients. Clin Microbiol Rev. 2002; 15(1): 145-154. https://dx.doi.org/10.1128/CMR.15.1.145-154.2002
2. Dong S, Yang Y, Wang Y, Yang D, Yang Y, Shi Y, et al. Prevalence of *Cryptosporidium* Infection in the Global Population: A Systematic Review and Meta-analysis. Acta Parasitol. 2020; 65(4): 882-889. https://dx.doi.org/10.2478/s11686-020-00230-1
3. Hadi AM. Isolation and Identification of *Cryptosporidium* sp. by Reverse Osmosis System of Tap water in Baghdad. Baghdad Sci J. 2014; 11(2): 894-899.
4. Abdul-Maksoud HF, Ismail MAM, Amer NM, EL Akkad DMH, Magdy M. Detection of *Cryptosporidium*-induced intestinal tissue alterations in Dexamethasone treated & un-treated mice. Egypt Soc Parasitol. 2018; 2(2): 227-249. https://doi.org/10.12816/0050453
5. Mccole DF, Eckmann L, Laurent F, Kagnoff MF. Intestinal epithelial cell apoptosis following *Cryptosporidium parvum* infection. Infect Immun. 2000; 68(3): 1710-1713. https://dx.doi.org/10.1128/IAI.68.3.1710-1713.2000
6. Benamrouz S, Conseil V, Chabé M, Praet M, Audebert C, Blervaque R, et al. *Cryptosporidium parvum*-induced ileo-caecal adenocarcinoma and Wnt signaling in a mouse model. DMM Dis Model Mech. 2014; 7(6): 693-700. https://dx.doi.org/10.1242/dmm.013292
7. Bhalchandra S, Lamisere H, Ward H. Intestinal organoid/enteroid-based models for *Cryptosporidium*. Curr Opin Microbiol. 2020; 58: 124-129. https://dx.doi.org/10.1016/j.mib.2020.10.002
8. Dhal A, Chinmaya P, Yun S, Mahapatra RK. An update on *Cryptosporidium* biology and therapeutic avenues. J Parasit Dis. 2022; 46(3): 923-939. https://dx.doi.org/10.1007/s12639-022-01510-5
9. Srikala SV, Santhi Priya N, Nadendla RR. Formulation, Characterization and Antihelminthic Activity Testing of Nitazoxanide Superporous Hydrogel Tablets. J Drug Deliv Ther. 2020; 10(3-s): 26-36. https://dx.doi.org/10.22270/jddt.v10i3-s.4130
10. Moawad HSF, Hegab MHA, Badawey MSR, Ashoush SE, Ibrahim SM, Ali AAELS. Assessment of chitosan nanoparticles in improving the efficacy of nitazoxanide on cryptosporidiosis in immunosuppressed and immunocompetent murine models. J Parasit Dis. 2021; 45(3): 606-619. https://dx.doi.org/10.1007/s12639-020-01337-y
11. Wuqing O, Jianjun G, Qiaoyan S. Oil-in-water-type compound nitazoxanid nanoemulsion and preparation method thereof. Published online 2012. https://patents.google.com/patent/CN102526039A/en
12. Salas-z R, Rodríguez-Ruiz C, Höpfl H, Morales-rojas H, Obdulia S, Rodr P. Dissolution Advantage of Nitazoxanide Cocrystals in the Presence of Cellulosic Polymers. Pharm Res. 2019; 12(1): 23. https://doi.org/10.3390/pharmaceutics12010023
13. Zoller U. Handbook of Detergents, Part E: Applications. 1st ed. CRC Press. New York; 2008. 141: 455-468..
14. Burggraaf W, Leng RA. Antiprotozoal effects of surfactant detergents in the rumen of sheep. New Zeal J Agric Res. 1980; 23(3): 287-291. https://dx.doi.org/10.1080/00288233.1980.10425358
15. Rangel-Yagui C de O, Pessoa A, Tavares LC. Micellar solubilization of drugs. J Pharm Pharm Sci. 2005; 8(2): 147-163.
16. Torchilin VP. Micellar nanocarriers: Pharmaceutical perspectives. Pharm Res. 2007; 24(1): 1-16. https://dx.doi.org/10.1007/s11095-006-9132-0
17. Vinarov Z, Katev V, Radeva D, Tcholakova S, Denkov ND. Micellar solubilization of poorly water-soluble drugs: effect of surfactant and solubilizate molecular structure. Drug Dev Ind Pharm. 2018; 44(4): 677-686. https://dx.doi.org/10.1080/03639045.2017.1408642
18. Bin-Hudayb NS, Badr EE, Hegazy MA. Adsorption and corrosion performance of new cationic gemini surfactants derivatives of fatty amido ethyl aminium chloride with ester spacer for mild steel in acidic solutions. Materials (Basel). 2020; 13(12): 1-28. https://dx.doi.org/10.3390/ma13122790
19. Sonntag NOV. Reactions of fatty acid chlorides: II. Synthesis of monohydrazides or dihydrazides by

acylation of hydrazine hydrate with saturated fatty acid chlorides. J Am Oil Chem Soc. 1968; 45(8): 571-574. https://dx.doi.org/10.1007/BF02667173
20. Kandeel EM, Badr EE, El-Sadek BM. Novel gemini cationic surfactants based on N, N-dimethyl fatty hydrazide and 1,3-dibromopropane: Synthesis, evaluation of surface and antimicrobial properties. J Oleo Sci. 2010; 59(12): 647-652. https://dx.doi.org/10.5650/jos.59.647
21. Quah JX, Ambu S, Lim YAL, Mahdy MAK, Mak JW. Molecular identification of *Cryptosporidium parvum* from avian hosts. Parasitology. 2011; 138(5): 573-577. https://dx.doi.org/10.1017/S0031182010001691
22. Madbouly Taha N, Salah A. Yousof HA, El-Sayed SH, Younis AI, Ismail Negm MS. Atorvastatin repurposing for the treatment of cryptosporidiosis in experimentally immunosuppressed mice. Exp Parasitol. 2017; 181: 57-69. https://dx.doi.org/10.1016/j.exppara.2017.07.010
23. Garba A, Tohon Z, Sidiki A, Chippaux JP, Chabalier F de. Efficacité et tolérance du praziquantel chez l'enfant d'âge scolaire en zone hyper-endémique à *Schistosoma haematobium* (Niger, 1999). Bull Soc Pathol Exot. 2001; 94(1): 42-45. http://dx.doi.org/10.3390/pharmaceutics12010023
24. Carter KC, Baillie AJ, Mullen AB. The cured immune phenotype achieved by treatment of visceral leishmaniasis in the BALB/c mouse with a nonionic surfactant vesicular formulation of sodium stibogluconate does not protect against reinfection. Clin Diagn Lab Immunol. 1999; 6(1): 61-65. https://dx.doi.org/10.1128/cdli.6.1.61-65.1999
25. Barnes JM, Paget GE. 2 Mechanisms of Toxic Action. Prog Med Chem. 1965; 4(C): 18-38. https://dx.doi.org/10.1016/S0079-6468(08)70166-8
26. Rasmussen KR, Healey MC. Experimental *Cryptosporidium parvum* infections in immunosuppressed adult mice. Infect Immun. 1992; 60(4): 1648-1652. https://dx.doi.org/10.1128/iai.60.4.1648-1652.1992
27. Tarazona R, Blewett DA, Carmona MD. *Cryptosporidium parvum* infection in experimentally infected mice: Infection dynamics and effect of immunosuppression. Folia Parasitol (Praha). 1998; 45(2): 101-107.
28. Henriksen S A, Pohlenz JFL. Staining of cryptosporidia by a modified Ziehl-Neelsen technique. Acta Vet Scand. 1981; 22: 594-596. https://dx.doi.org/10.1186/BF03548684
29. Penido MLDO, Nelson DL, Vieira LQ, Coelho PMZ. Schistosomicidal Activity of Alkylaminooctanethiosulfuric Acids. Mem Inst Oswaldo Cruz. 1994; 89(4): 595-602. https://doi.org/10.1590/S0074-02761994000400017
30. Day CE. Histopathology: Methods and Protocols. 1st ed Humana Press; 2014. 1180: 31-43. https://link.springer.com/book/10.1007/978-1-4939-1050-2
31. Hui E. Learn R for Applied Statistics With Data Visualizations, Regressions, and Statistics. 1st ed. APress; 2019. 173-236.
32. Labena A, Hegazy MA, Horn H, Müller E. Sulfidogenic-corrosion inhibitory effect of cationic monomeric and gemini surfactants: Planktonic and sessile diversity. RSC Adv. 2016; 6(48): 42263-42278. https://dx.doi.org/10.1039/c6ra02393b
33. Labena A, Hegazy MA, Kamel WM, Elkelish, A. &, Hozzein WN. Enhancement of a cationic surfactant by capping nanoparticles: Synthesis, characterization and multiple applications. Molecules. 2020; 25(9): 2007. https://doi.org/10.3390/molecules25092007
34. Ezzat AO, Tawfeek AM, Al-Lohedan HA. Synthesis and application of novel gemini pyridinium ionic liquids as demulsifiers for arabian heavy crude oil emulsions. Colloids Surfaces A Physicochem Eng Asp. 2022; 634: 127961. https://dx.doi.org/10.1016/j.colsurfa.2021.127961
35. Pillai P, Pal N, Mandal A. Synthesis, characterization, surface properties and micellization behaviour of imidazolium-based ionic liquids. J Surfactants Deterg. 2017; 20(6): 1321-1335. https://doi.org/10.1007/s11743-017-2021-1
36. Alazrak SM, Awad S, Khalil AA, El-Dougdoug W. Synthesis and evaluation of new cationic polymeric surfactant based on n-phthalimidomethyl methacrylate. Egypt J Chem. 2021; 64(7): 3861-3872. https://dx.doi.org/10.21608/ejchem.2021.54791.3224
37. Kareem SH, Zead LA. CMC Determination and Thermodynamic Micellisation Of NPE Surfactant In Aqueous And CH 3 OH – H 2 O Solvents. Baghdad Sci J. 2013; 10(3): 1050-1056.
38. Moulik SP, Rakshit AK, Naskar B. Evaluation of Non-Ambiguous Critical Micelle Concentration of Surfactants in Relation to Solution Behaviors of Pure and Mixed Surfactant Systems: A Physicochemical Documentary and Analysis. J Surfactants Deterg. 2021; 24(4): 535-549. https://dx.doi.org/10.1002/jsde.12503
39. Azum N, Rub MA, Asiri AM, Bawazeer WA. Micellar and interfacial properties of amphiphilic drug–non-ionic surfactants mixed systems: Surface tension, fluorescence and UV–vis studies. Colloids Surfaces A Physicochem Eng Asp. 2017; 522: 183-192. https://dx.doi.org/10.1016/j.colsurfa.2017.02.093
40. Bhadani A, Endo T, Sakai K, Sakai H, Abe M. Synthesis and dilute aqueous solution properties of ester functionalized cationic gemini surfactants having different ethylene oxide units as spacer. Colloid Polym Sci. 2014; 292(7): 1685-1692. https://dx.doi.org/10.1007/s00396-014-3233-9
41. Bhadani A, Tani M, Endo T, Sakai K, Abe M, Sakai H. New ester based gemini surfactants: the effect of different cationic headgroups on micellization properties and viscosity of aqueous micellar solution. Phys Chem Chem Phys. 2015; 17(29): 19474-19483. https://dx.doi.org/10.1039/c5cp02115d
42. Rosen MJ, Kunjappu JT. Surfactants and Interfacial Phenomena. 4th ed WILEY. New Jersey; 2012: 1-38. https://dx.doi.org/10.1002/9781118228920
43. Puvvada S, Blankschtein D. Molecular-thermodynamic approach to predict micellization, phase behavior and phase separation of micellar solutions. I. Application to nonionic surfactants. J

Chem Phys. 1990; 92(6): 3710-3724. https://dx.doi.org/10.1063/1.457829
44. Torchilin VP. Structure and design of polymeric surfactant-based drug delivery systems. J Control Release. 2001; 73(2-3): 137-172. https://dx.doi.org/10.1016/S0168-3659(01)00299-1
45. Krishna AK, Flanagan DR. Micellar solubilization of a new antimalarial drug, β-arteether. J Pharm Sci. 1989; 78(7): 574-576. https://dx.doi.org/10.1002/jps.2600780713
46. Mosmann T. Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays. RSC Adv. 1983; 6(65): 55-63. https://dx.doi.org/10.1016/0022-1759(83)90303-4
47. Abd-El-Aziz AS, El-Ghezlani EG, Elaasser MM, Afifi TH, Okasha RM. First Example of Cationic Cyclopentadienyliron Based Chromene Complexes and Polymers: Synthesis, Characterization, and Biological Applications. J Inorg Organomet Polym Mater. 2020; 30(1): 131-146. https://dx.doi.org/10.1007/s10904-019-01295-w
48. El-Badry AA, Abdel Aziz IZ, Shoeib EY, Ghallab MMI. *Cryptosporidium* genotypes and associated risk factors in a cohort of Egyptian children. Comp Clin Path. 2017; 26(5): 1017-1021. doi:10.1007/s00580-017-2477-4
49. Pumipuntu N, Piratae S. Cryptosporidiosis: A zoonotic disease concern. Vet World. 2018; 11(5): 681-686. https://dx.doi.org/10.14202/vetworld.2018.681-686
50. Wang R jun, Li J qiang, Chen Y cai, Zhang L xian, Xiao L hua. Widespread occurrence of *Cryptosporidium* infections in patients with HIV/AIDS: Epidemiology, clinical feature, diagnosis, and therapy. Acta Trop. 2018; 187: 257-263. https://dx.doi.org/10.1016/j.actatropica.2018.08.018
51. Sosnik A, Augustine R. Challenges in oral drug delivery of antiretrovirals and the innovative strategies to overcome them. Adv Drug Deliv Rev. 2016; 103: 105-120. https://dx.doi.org/10.1016/j.addr.2015.12.022
52. Kanoje B, Patel D, Kumar V, Sahoo SK, Parikh J, Kuperkar K. Unraveling the solubilization and cytotoxicity study of poorly water-soluble anti-inflammatory drug in aqueous Gemini surfactants solution with physicochemical characterization and simulation study. Colloids Surfaces B Biointerfaces. 2019; 179: 437-444. https://dx.doi.org/10.1016/j.colsurfb.2019.03.059
53. Fatma N, Panda M, Ansari WH. Colloids and Surfaces A : Physicochemical and Engineering Aspects Solubility enhancement of anthracene and pyrene in the mixtures of a cleavable cationic gemini surfactant with conventional surfactants of different polarities. Colloids Surfaces A Physicochem Eng Asp. 2015; 467: 9-17. https://dx.doi.org/10.1016/j.colsurfa.2014.11.010
54. Gaggero A, Jurišić Dukovski B, Radić I, et al. Co-grinding with surfactants as a new approach to enhance in vitro dissolution of praziquantel. J Pharm Biomed Anal. 2020; 189: 1-12. https://dx.doi.org/10.1016/j.jpba.2020.113494
55. Kolenyak-Santos F, Garnero C, De Oliveira RN, De Souza AL, Chorilli M, Allegretti SM, et al. Nanostructured lipid carriers as a strategy to improve the in vitro schistosomiasis activity of Praziquantel. J Nanosci Nanotechnol. 2015; 15(1): 761-772. https://dx.doi.org/10.1166/jnn.2015.9186
56. Abdelhamed E, Fawzy E, Ahmed S, Zalat R, Rashed H. Effect of Nitazoxanide, Artesunate Loaded Polymeric Nano Fiber and Their Combination on Experimental Cryptosporidiosis. Iran J Parasitol. 2019; 14(2): 240-249.
57. Fahmy M-E, Abdelaa A, Hassan S, Shalaby M, Ismail MA, Khairy R, et al. Antiparasitic and immunomodulating effects of nitazoxanide , ivermectin and selenium on *Cryptosporidium* infection in diabetic mice. Rev Bras Parasitol Veterinária. 2021; 30(4): 1-16. https://dx.doi.org/10.1590/S1984-29612021087
58. Sadek GS, El-Aswad BE. Role of COX-2 in pathogenesis of intestinal Cryptosporidiosis and effect of some drugs on treatment of infection. Res J Parasitol. 2014; 9(2): 21-40. https://dx.doi.org/10.3923/jp.2014.21.40
59. El-wakil ES, Salem AE, Al-Ghandour AM. Evaluation of possible prophylactic and therapeutic effect of mefloquine on experimental cryptosporidiosis in immunocompromised mice. J Parasit Dis. 2021; 45(2): 380-393. https://dx.doi.org/10.1007/s12639-020-01315-4
60. Certad G, Benamrouz S, Guyot K, Mouray A, Chassat T, Flament N, et al. Fulminant Cryptosporidiosis after Near-Drowning : a Human *Cryptosporidium* parvum Strain Implicated in Invasive Gastrointestinal Adenocarcinoma and Cholangiocarcinoma in an Experimental Model. Appl Environ Microbiol. Published online 2012: 1746-1751. https://dx.doi.org/10.1128/AEM.06457-11
61. Metawae A, Bayoumy A, Ali I, Hammam O, Temsah K. Efficacy of Nitazoxanide Alone or Loaded with Silica Nanoparticle for Treatment of Cryptosporidiosis in Immunocompetent Hosts. Int J Med Arts. 2021; 3(1): 1229-1239. https://dx.doi.org/10.21608/ijma.2021.55788.1237

Open Access **Baghdad Science Journal** **P-ISSN: 2078-8665**
Published Online First: April, 2023 **2023, 20(6): 2086-2105** **E-ISSN: 2411-7986**

تقييم كفاءة النيتازوكسانيد مع مواد ذات نشاط سطحي توأمية موجبة في الجسم الحي على داء الكريبتوسبوريديوسس

زينب أحمد[1] إيمان محمد قنديل[1] هاجر فتحي عبد المقصود[2]* طارق أبو شوشة[3] انتصار السيد بدر[1]

[1]كلية العلوم للبنات، جامعة الأزهر، القاهرة، مصر.
[2]قسم علم الطفيليات، معهد ثيودور بلهارس للأبحاث، الجيزة، مصر.
[3]قسم علم الأمراض، معهد ثيودور بلهارس للأبحاث، الجيزة، مصر.

الخلاصة:
تُعرض الإصابة بداء خفيات الأبواغ حياة العديد من الأشخاص للخطر وخصوصا المصابين بنقص المناعة، تحديدا مرضى فيروس نقص المناعة البشرية . يُعد النيتازوكسانيد أحد الأدوية العلاجية الرئيسية المستخدمة في علاج داء الكريبتوسبوريديوسس. ومع ذلك، فهو ضعيف الذوبان في الماء ، مما يحد من فائدته وفعاليته في المرضى الذين يعانون من نقص المناعة. يحتوي الفاعل بالسطح على طابع برمائي وهذا يشير إلى قدرتها على تحسين قابلية الذوبان في الماء للعقار المضاد للماء. يتعلق بحثنا بتركيب مواد خافضة للتوتر السطحي من الجوزاء الموجبة الجديدة والتي لديها القدرة على تحسين قابلية ذوبان عقار نانازوكسيد. لذلك قمنا بتوليف مواد خافضة للتوتر السطحي توأمية موجبة. -N^1,N^1,N^3,N^3 tetramethyl-N^1,N^3-bis(2-octadecanamidoethyl)propane-1,3-diaminium bromide (CGSPS18) و 2,2`-(ethane-1,2-diylbis(oxy))bis(N-(2-octadecanamidoethyl)-N,N-dimethyl-2-oxoethane-1-aminium) dichloride (CGSES18) وتأكيد تركيبها الكيميائي بالطرق الطيفية المختلفة وكذلك دراسة خصائص السطح والسمية لها. بالإضافة إلى ذلك، تمت دراسة فعالية نيتازوكسانيد في الفئران المصابة بإضافة ثلاث جرعات مختلفة من المواد الخافضة للتوتر السطحي. لمعرفة تأثير النيتازوكسانيد والمواد الخافضة للتوتر السطحي معا، تم حساب العدوى بالطفيليات قبل العلاج وبعده ، كما تم فحص الأنسجة المعوية والكبدية والرئوية. في هذه الدراسة وجد أن الجمع بين عقار نيتازوكسانيد مع المواد الخافضة للتوتر السطحي وخاصة المركب (CGSPS18) بتركيز 25٪ زاد من الفعالية وأدى إلى انخفاض بنسبة 90.8٪. أظهر فحص الأنسجة المرضية أن المجموعة التي عولجت بعقار نيتازوكسانيد مع CGSPS18 أظهرت أفضل النتائج التي أظهرت نمطًا زغبيًا طبيعيًا تقريبًا. أظهرت هذه الدراسة زيادة في فعالية النيتازوكسانيد عند دمجه مع المواد الخافضة للتوتر السطحي ، وهذا يشير إلى مستقبل واعد لاستخدام المواد الخافضة للتوتر السطحي كعامل مساعد لتعزيز فعالية النيتازوكسانيد في علاج داء خفيات الأبواغ في المرضى الذين يعانون من نقص المناعة ، وخاصة مرضى فيروس نقص المناعة البشرية.

الكلمات المفتاحية: خافض التوتر السطحي التوأمي الموجب، خفية الأبواغ، مرضى نقص المناعة البشرية، تنقص المناعة، نيتازوكسانيد.