

Micellar Properties and Antimicrobial Activity of a Mixed Surfactant System Constituted by Sodium Dodecyl Sulfate and Cetylpyridinium Chloride

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ABSTRACT

Objective: Micellar properties of sodium dodecyl sulfate (NaDS) was examined in the presence of cetylpyridinium chloride (CPC) by means of surface tension, viscosity, dye solubilization, cloud point (CP) measurements. Antimicrobial activities of single and binary systems were also investigated. **Results:** A decrease in critical micelle concentration (CMC) and increase in solubilizing power for NaDS was observed with increasing CPC concentration. At the highest CPC concentration studied (0.1 M), the surface tension decreased up to 51 mN / m. Also, viscosity results showed growth in NaDS micelles (50 mM) up to the 0.1 M CPC concentration. Antimicrobial activity of NaDS, CPC and NaDS in presence of CPC was investigated against ten different strains of bacteria and yeast which was not investigated up to now. The susceptibilities of the microorganism were determined by the agar diffusion method. The results showed that NaDS and CPC have an antimicrobial activity but NaDS in presence of CPC has not antimicrobial activity on the bacterial and yeast.

Key words: Mixed Micellization, NADS, CPC, Antimicrobial Activity.

INTRODUCTION

Surfactants are widely used in different applications such as detergents, foaming and antifoaming agents, cosmetics, floatation agents and pharmaceuticals etc.¹ The detergents and personal care products use nearly 60% of all surfactants. The biggest advantage of surfactants is their distinct toxic activity towards organisms due to their surface activity. Their choice depends on many factors among which solubility of the surfactants and their CMC play an important role.² Anionic surfactants, particularly alkylbenzene sulfonates, are the most widely used surfactants in detergents and personal care products. Cationic surfactants are not used very often in personal care products because of their very high irritation properties in comparison to other surfactants.^{3,4} In hygiene products as well as in the cosmetic preparations require sur-

factants with low antibacterial activity and high emulsifying potential in order to help keep skin's flora and moisture level in balance.⁵ The commonly used cationic surfactants include benzylalkylammonium, alkyl quaternary ammonium, alkylpyridinium salts. Quaternary ammonium surfactants (QAS) are effective classes of antimicrobial agents and are very widely used in cosmetics, antiseptics, hospital sanitizers and contact lens disinfectants.^{6,7} Antimicrobial activity of QAS is a function of surfactant properties.⁸ These surfactants generally have a low surface tension and a higher adsorption efficiency at the interface.^{9,10} In particular, QASs with alkyl chains below a certain length, and so weak with surfactant properties, are ineffective as antimicrobial agents.¹¹ These properties provide their adsorption onto negatively charged bacterial surfaces. Their antibacterial properties were first

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described by Jacobs and Heidelberger in 1915 studying the antibacterial activity of substituted hexamethylenetetrammonium salts.^{12,13} Domagk synthesized long-chain QASs, including benzalkonium chloride, and characterized their antibacterial activities, that the second important step in the work of antibacterial QASs took place.¹⁴ In general, mixtures of different types of surfactants were employed for industrial purposes. Surfactant mixtures are well known to possess better chemical and surface-active properties than pure surfactants and thereby decreasing their required amount in applications.¹⁵⁻¹⁷ Most studies on mixed surfactant systems are concerned with physicochemical aspects such as CMC measurements, micelle formation, modeling etc., of these systems.¹⁸⁻²⁰ Mata *et al.*²¹ studied the micellar properties of NaDS in the presence of tetrabutylammonium bromide (TBABr) and concluded that the NaDS shows a remarkable decrease in surface tension, CMC and enhanced solubilization power in presence of TBABr. The aim of this study was to determine the stability and physicochemical and antimicrobial properties of mixed surfactant systems.

MATERIALS AND METHODS

The anionic surfactant sodium dodecyl sulfate (NaDS) and cationic surfactant cetylpyridinium chloride (CPC) were obtained from Fluka and were used without further purification.

Bacterial cells: *Aeromonas hydrophila* (106); *Yersinia enterocolitica* (ATCC 1501); *Pseudomonas aeruginosa* (ATCC 29212); *Escherichia coli* (ATCC 35218); *Bacillus subtilis* (ATCC 6633); *Bacillus cereus* (RSKK 863); *Staphylococcus aureus* (259231); *Micrococcus lutes* (NRL B-4375)

Yeast Cell: *Saccharomyces cerevisiae* T-32

Surface tension

The surface tension (ST) of NaDS solutions in absence and presence of CPC was measured by drop numbers method using a stalagmometer (Traub's Stalagmometer Model 4855). Before using the stalagmometer was cleaned and dried and mounted in the vertical plane by using burette stand. In this process, first the stalagmometer was filled with distilled water as above without changing the pressure. Using the screw pinch cork, the flow rate was adjusted to 10 drops/min. The number of drops of water was counted between the marks of the stalagmometer (n_1). Water was removed and the stalagmometer was filled with NaDS solution containing CPC in concentration 0-100 mM and number of drops was counted (n_2). The process was repeated three times and

surface tensions were determined using formula given below.

$$ST \text{ of solution } \gamma_1 = \gamma_2 (n_2/n_1) \cdot (d_1/d_2)$$

where n_1 = Number of drops of solutions

d_1 = Density of solution at room temperature

γ_2 = Surface tension of water at room temperature (72.8 mN/m)

n_2 = Number of drops of water

d_2 = Density of water at room temperature (0.99820 g/mL; 20°C)

Dye solubilization

For the dye solubilization experiments, a water insoluble dye, orange-G ($C_{16}H_{14}N_2Na_2O_7S_2$), mol wt. = 452.37) was used. The dye was shaken with an aqueous solution of the surfactant for 48 hours at room temperature and then the residue was removed by means of centrifugation and filtration. The absorbance of the resultant solution was then measured with an ultraviolet spectrophotometer (UV-6105) at 25°C.

Cloud point

Cloud point was determined at fixed concentration of the NaDS (50 mM) in the absence and presence of varying amount of added CPC (0-100 mM). Surfactant solution in thin 20 mL glass tubes stirred with a magnetic bar while being heated. The heating rate of the sample was controlled 1°C/min. The first appearance of turbidity was taken as the cloud point.

Viscosity

The viscosity measurements were carried out using an Ubbelohde suspended level capillary viscometer. The viscometer was always suspended vertically in a thermostat at 25±0.1°C. The viscometer was cleaned and dried every time before each measurements. The flow time for constant volume of solution through the capillary was measured with a calibrated stopwatch.

Antimicrobial activities

Microorganisms provided from the culture collection of the Biotechnology Laboratory of the Science and Technology of Gazi University, TURKEY.

Bacterial cells and yeast cells were used as the test organisms in an antimicrobial study. Bacterial strains were inoculated into Nutrient Broth (Difco) and incubated at 30°C±1.0°C (for 24 h). In order to test antimicrobial effects of NaDS, CPC and NaDS in the presence of CPC and 15 mL of Mueller Hinton agar (Merck) were placed in petri dishes which were then inoculated with strains of bacteria by taking 100 mL from cell culture media. It was left to solidify at room temperature for

a while and then holes were made on top with a sterile stick. Test compounds prepared at the 20 mg/mL concentration. 50 mL of test compounds were added to the holes. Petri dishes were left at 4°C for 2 h. Then bacterial cultures were incubated at 37°C±1.0°C (for 24 h).

Yeast strain was inoculated into YBD Broth (Difco) and incubated at 25°C±1.0°C (for 48 h). In order to test antimicrobial effects of NaDS, CPC and NaDS in the presence of CPC and 15 mL of *YBD* agar were placed in petri dishes which were then inoculated with strain of bacteria by taking 100 mL from cell culture media. The same operations as mentioned above were as repeated. Yeast cultures incubated at 30°C±1.0°C (for 48 h). End the incubation time the inhibition zone of on the bacterial and yeast nutrient media were measured Table 1. Deionized water were used for control samples.

RESULTS AND DISCUSSION

Surface-active behavior of NaDS in absence and presence of CPC

Figure 1 shows surface-active behavior of NaDS in absence and presence of CPC concentrations. The surface tension of NaDS decreased with increasing of surfactant concentration up to CMC, beyond which no considerable change was noticed. This is a common behavior shown by surfactants in solution and is used to determine their purity and CMCs. The CMC value of CPC in water was found to be 9.0×10^{-4} M by Schamehorn.¹⁵ The alteration of electrical atmosphere of NaDS in the presence of CPC neutralizes the effective head group charge probably resulting in reduced electrostatic repulsion between the charged head groups and thereby tend to form micelles at much lower concentrations. The CMC of NaDS decreased in the presence of CPC, the decrease being dependent upon the concentration of CPC. Mixtures of different surfactant types always found to have lower CMCs and interfacial tensions than would be expected based on the properties of the pure surfactants.²² This situation leads to an increase in both theoretical and practical interest in developing a quantitative understanding of mixed surfactant behavior, and could be exploited in applications such as detergency,²³ enhanced oil recovery²⁴ and mineral flotation.²⁵ However, it is found that the correlation between the ability of a surface-active compound to lower surface tension and its ability to prevent microbial adhesion.²⁶

Solubility of dye orange-G in the surfactant micelles

One of most important properties of surfactants is their solubilizing power. To measure solubilizing behavior

of surfactants, the solubilization of water insoluble dye orange-G in the surfactant micelles was studied. When the absorbance versus concentration of the NaDS was plotted, the linear relationships shown in Figure 2 were obtained. The absorbance for pure surfactant increases with increase in concentration of surfactant NaDS. The results show that the amount of dye solubilized was insignificant up to the CMC of NaDS and thereafter a sudden steep increase was observed with the formation of micellised surfactant in the bulk. It is evident that the solubilizing power of NaDS increases in the presence of CPC. This increase is due to the diluted of micellar surface charge density leading to micelle swelling. The CMC so determined is in good agreement with the value obtained by surface tension technique. Variations in CMC values depending on the method of determination have been reported in literature.²⁷

Variation of cloud point with NaDS in the presence of CPC

Figure 3 shows the variation of cloud point with NaDS (50 mM) in the presence of different concentrations of CPC. The CP decreases with increase in CPC concentration. The cloud point of a surfactant is an important factor to be considered at screening surfactant applications, because considerable changes in physical properties and, hence, in the performance of a surfactant solution is expected in temperature near the cloud point of the solution. It is well known that CP is characteristic property of nonionic surfactants while in case of ionic surfactants, the phenomenon is rarely observed.^{28,29} At the cloud point, the water molecule gets very detached from micelles. For charged micelles, occurrence of the phenomenon is rarer, because presumably electrostatic repulsion prevents phase separation in most cases. Above its cloud point temperature or in the presence of certain additives, the aqueous nonionic surfactant solutions separate into two phases, a dilute phase and a surfactant-rich phase which is called cloud point system (CPS). The CPS is an attractive system because it provides a separation technique which is simple to operate, easy to manipulate and reliable to scale up. Especially, it provides an aqueous medium so that microbial cells will be protected from damage.^{30,31} The inhibition or toxicity of both substrate and product may be reduced and the biocompatibility may be increased in cloud point system. Surfactants increase apparent aqueous solubility of hydrophobic organic compounds (known as solubilization) and are used to improve the bioavailability and biodegradation of these contaminants to.^{32,33} The experimental results show that the cloud point of NaDS is helpful to exploit a biocompatible medium for a microbial growth

and then for whole cell microbial transformation in a nonaqueous medium.

Temperature and component concentration can affect the stability of the mixed micelles i.e., micelles destabilized at lower temperatures with increasing encapsulated component. For example, 0.9% of eugenol encapsulated in Surfynol® 485W exhibited turbidity (cloud point) at 55°C, while at 0.5%, 70°C was required to reach the cloud point. At temperatures optimal for microbial growth, micelles were stable and retained activity.³⁴

The CP of an amphiphile can be considered as the limit of its solubility as it phase separates at temperatures above the CP. The dehydration of the surfactant's hydrophilic groups leads to the phase separation and the formation of cloudy dispersion and it reduces repulsive interactions which maintain micelles as discrete units and thus facilitates micellar growth. Many theories were put forward to explain the occurrence of CP; it is still not completely resolved.³⁵⁻³⁷

The solution's cloud point is affected by the presence of other components in a formulation. The CPs of the mixtures of the surfactants were found to be in between the CP of individual component surfactant. Van der Waals attraction and penetration effect will help in attraction two micelles together, while the electrostatic repulsion will prevent the micellar contact. More CPC concentration will replace more structured water and phase separation is expected to appear at a lower temperature since the NaDS concentration is constant. This is clearly reflected from the Figure 3.

Determination of relative viscosity of NaDS as a function of CPC

Figure 4 shows that relative viscosity (μ) of NaDS (50 mM) as a function of CPC. Relative viscosity (μ) of NaDS (50 mM) increased with increasing CPC concentration.

The formation of micelle aggregates structure from surface active molecules is governed by a delicate balance between the attractive and repulsive interactions of the surface free energy. In the mixtures of the surfactants viscosity show deviation from ideal behavior because of the electroviscous effect. If the mixed micelles form easily, the electroviscous effect will be large. As the CPC salts used in this study contain a positive charge on the N-atom which will decrease the effective charge of the NaDS and replacement of water by alkyl chains will be responsible for micellar growth.

Antimicrobial Activity of Surfactants in Pure and Mixed Micelles

Table 1 was shown that CPC and NaDS have almost been same antimicrobial activity studied concentration. Mixed of CPC and NaDS have not shown antimicrobial activity against studied microorganisms except *Aeromonas hydrophila* (106). Surfactants can inhibit the development of microorganisms in different ways. Foght *et al.* reported that the emulsifier, Emulsan, stimulated aromatic mineralization by pure bacterial cultures, but inhibited the degradation process when mixed cultures were used.³⁸ Ito *et al.* reported that the sophorolipids inhibited on growth of yeast on water-insoluble substrates.^{39,40} According to Paul and Jeffrey (1985), dilute surfactants completely inhibited the attachment of estuarine and marine bacteria.⁴¹ They can destroy the structure of cell and inhibit some enzymes and can destroy DNA or stop the development of microorganisms by inhibition of protein synthesis. Destruction due to surfactants is the result of preferred partitioning of surfactants from the aqueous phase into cell membranes where, at low concentrations, they affect some physical properties (pressure, surface charge, etc.) which then can significantly affect a membrane protein's functions,

Table 1: The antimicrobial activities of NaDS, CPC and NaDS in the presence of CPC on the bacterial and yeast cells (inhibition zone = mm)

Bacterial Strain	NaDS	CPC	NaDS-CPC	Bacterial Strain	NaDS	CPC	NaDS-CPC
<i>Aeromonas Hydrophila</i> 106	5	5	10	<i>Micrococcus Luteus</i> (NRLL B-4375)	10	10	-
<i>Esherichia Coli</i> (ATCC 35218)	4	4	6	<i>Staphylococcus Aureus</i> (ATCC 259231)	8	10	-
<i>Pseudomonas aerogonisa</i> (ATCC 9212)	-	6	-	<i>Yercinia Enterecolitice</i> (ATCC 1501)	6	-	-
<i>Bacillus cereus</i> (RSKK 863)	9	8	-	<i>Basillus subtilis</i> (ATCC6633)	8	10	-
Yeast Strain							
<i>Saccaromyces cerevisiae</i> T-32	10	-	-				

without a gross destruction of the membrane. At higher concentrations, closer to the surfactant CMC, an equilibrium is established between the cell membrane components associated with the lipid bilayer phase and a coexisting micellar pseudophase in the aqueous medium that results in a dissolution of several components of the lipid bilayer into micelles, destruction of cell membrane integrity, and cell lysis.⁴²⁻⁴⁴

In this study, surfactants may follow one or more mechanisms which was mentioned above. Anionic surfactants themselves show marked biological activity too either binding to various bioactive macromolecules such as starch,⁴⁵ proteins,⁴⁶ peptides and DNA⁴⁷ or by inserting into various cell fragments (i.e. phospholipid membranes) causing malfunction.

It is mentioned that NaDS in the presence of CPC will stimulate development of microorganisms and surfactant inhibition effect of CPC is greater than SDS. Surfactants can activate or inhibit the enzyme depending

on the surfactant concentration and on the length of alkyl chain. Cationic surfactant tested in this study was CPC greatly inhibited the enzyme activity even at the lowest concentration. All of the cationic surfactants demonstrate good surface-active properties and antibacterial activity therefore they show potential applications in medical fields. Activity depends on the type and length of substituent at the quaternary nitrogen atom.^{48,49} Through hydrophobic interaction by their non-polar tail, these surfactants can disrupt native conformation of the enzyme. The non-polar tail subsequently interacts with the hydrophobic membrane core. At concentrations normally used for application to surfaces, cationic surfactants form mixed-micelle aggregates with hydrophobic membrane components that solubilize membrane and lyse the cells. Anionic surfactant, such as SDS is an anionic surfactant used in many cleaning and hygiene products and is shown by several studies to inhibit bacterial biofilm formation and disperse mature biofilms.

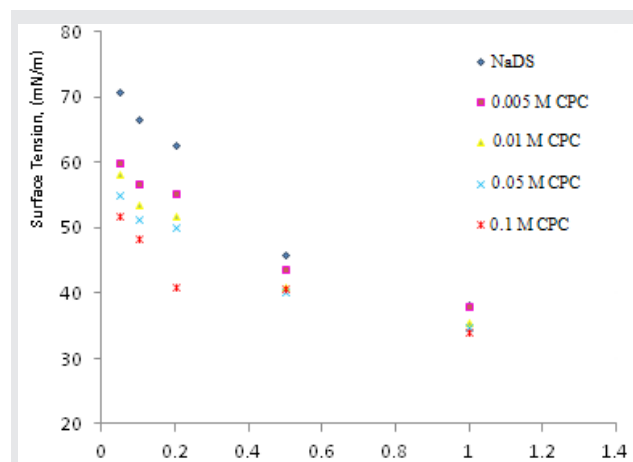


Figure 1: Surface activation behaviour of NaDS in absence and presence of CPC.

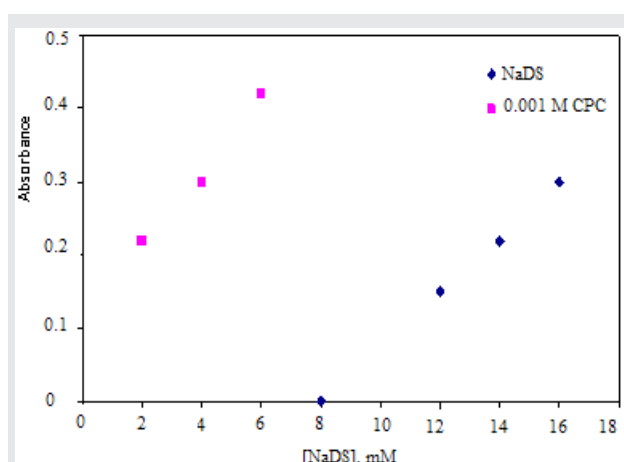


Figure 2: Absorbance of NaDS in absence and presence of CPC.

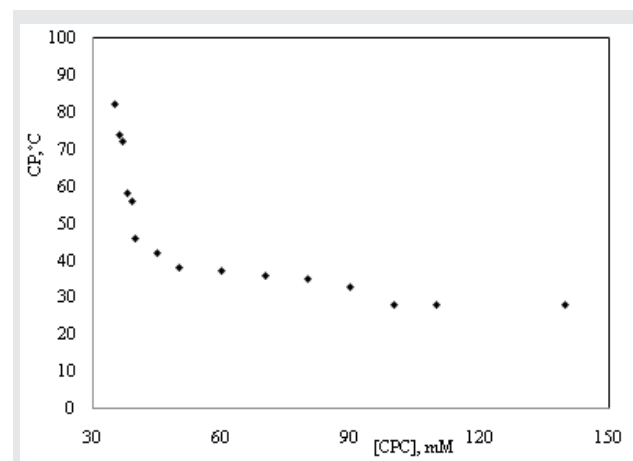


Figure 3: Cloud point of NaDS (50 mM) as a function of CPC.

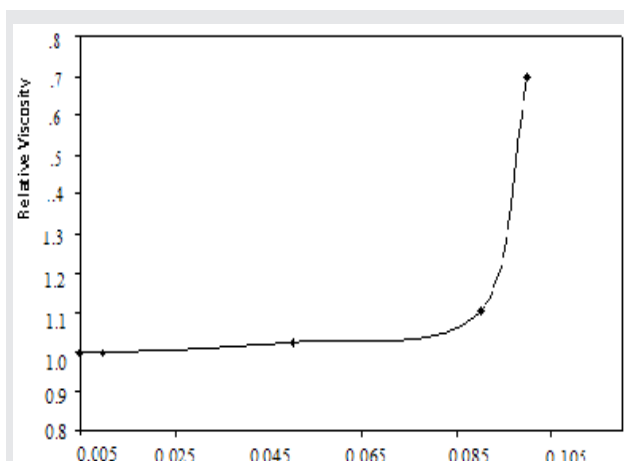


Figure 4: Relative viscosities (μ) of NaDS as a function of CPC.

SDS had minimal influence on enzymatic activity when it was tested under the CMC (0.1 mg/mL). However, at CMC and above, SDS completely inhibited the enzyme. Moreover, as in the case of cationic surfactants, charge interactions are the primary reason for the inhibition of enzymatic activity.

NaDS inhibited the ATPase activity of P-glycoprotein at very low concentrations while Triton X-100 stimulated at low concentration and inhibited the activity at higher concentrations.⁵⁰ Both sodium dodecyl sulfate (NaSDS) and, dodecyltrimethyl ammonium bromide (DTAB) caused inhibition of lecithin/cholesterol acyltransferase with a water-soluble substrate, whereas the nonionic surfactant Triton X-100, activated the enzyme.⁵¹ NaDS and DTAB modified the structure and enzymatic activity of jack bean urease⁵² and NaDS activated latent potato leaf polyphenol oxidase.⁵³ It was found that the interaction between the peptide and NaDS is of exclusively electrostatic character with the six positively charged arginines of the peptide acting as binding sites for NaDS. This binding may explain that similar to other as binding sites for NaDS⁵⁴ surfactants show *in vitro* antiviral activity against HIV-1, HIV-2 and other enveloped viruses.⁵⁵ Anionic surfactants influence enzyme activities has been extensively demonstrated. Thus, it was proved that linear alkyl benzene sulfonate can accumulate in the hepatic liposomes of the rat and can inhibit the activity of the enzymes alkaline phosphatase and acid phosphatase.⁵⁶

CONCLUSION

Micellar and antimicrobial behavior of NaDS and CPC mixtures in aqueous media has been investigated with help of surface tensiometry, dye solubilization, viscosity, CP, antimicrobial activities. NaDS shows a remarkable decrease in surface tension, CMC and enhanced solubilization power in the presence of CPC. Clouding phenomena was observed at room temperature in NaDS-CPC systems. Studied bacteria are recognized as human pathogens. We can say that both NaDS and CPC can use in the application against studied microorganisms.

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CONFLICT OF INTEREST

There is no conflict of interest.

ABBREVIATIONS USED

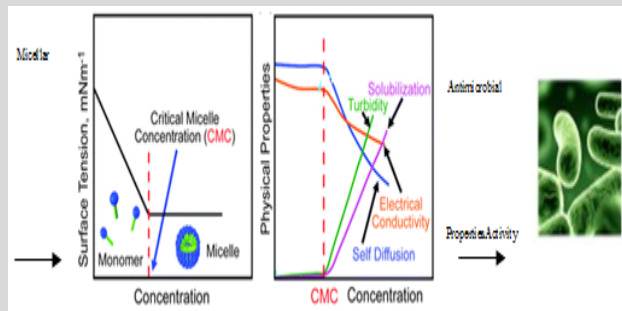
CMC: Critical micelle concentration; **CPC:** Cetylpyridinium chloride; **CP:** Cloud point; **CPS:** Cloud point system; **d₁:** Density of solution at room temperature; **d₂:** Density of water at room temperature (0.99820 g/mL; 20°C); **n₁:** Number of drops of solutions; **n₂:** Number of drops of water; **γ₂:** Surface tension of water at room temperature (72.8 mN/m); **μ:** Relative viscosity; **NaDS:** Sodium dodecyl sulfate; **QAS:** Quaternary ammonium surfactants; **TBABr:** Tetrabutylammonium bromide.

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PICTORIAL ABSTRACT



- A decrease in critical micelle concentration (CMC) and increase in solubilizing power for NaDS was observed with increasing CPC concentration.
- Both NaDS and CPC can use in the application against studied microorganics.

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