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Cationic Gemini Surfactants with Pyridinium Headgroup: Recent Advances

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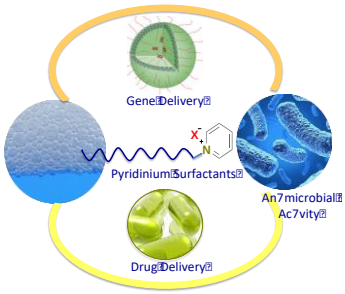
Gemini surfactants are comparatively superior surfactant molecules compared to their monomeric analogues. In recent years several new structural derivatives of these surfactants have been developed, pyridinium gemini surfactants are one of them. These new group of cationic gemini surfactants are being considered as potential surfactant molecule for several application area ranging from colloid science to biological science. The current review article emphasizes on recent advances and physicochemical properties of gemini pyridinium surfactants which have established themselves as future potential surfactant molecule for wide range of applications.



Keyword: *Gemini Pyridinium Surfactants; Self-Aggregation, Properties; Antimicrobial Activity; Gene Delivery; Drug Interaction.*

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Avinash Bhadani received his PhD degree from Guru Nanak Dev University in 2012 under supervision of Prof. Sukhprit Singh. Currently he is working as Postdoctoral Researcher under supervision of Prof. Masahiko Abe in Tokyo University of Science. His research interest includes synthesis of new type of gemini surfactants, investigation of their self-aggregation properties in aqueous system and evaluation of the physicochemical properties of surfactants in presence of different additives.

Masahiko Abe received his PhD degree from University of Tokyo, long time ago. He is currently Professor at Research Institute for Science and Technology, Tokyo University of Science. His research work is focused towards interdisciplinary sciences related to surfactant science such as synthesis and properties of hybrid surfactant, gemini surfactant, colloidal-assemblies (micro emulsion, emulsion, liposomes, niosomes, micro capsules, liquid crystals). Currently he is investigating gemini surfactants for various application, novel liposomes for non-DDS fields (upgrading of various polymers), and on development of glucose fuel battery.

Cationic Gemini Surfactants with Pyridinium Headgroup: Recent Advances

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1. Introduction: Twin tail and twin headgroup containing surfactants often termed as 'gemini surfactants' are the novel surfactant molecules which demonstrates comparatively better surface and biological properties compared to their monomeric derivatives.[1,2] Positively charged gemini surfactants are important class of surfactant which are currently being extensively investigated for their unique physicochemical properties and ability to interact with several negatively charged material/molecules/surfaces.[3-6] Gemini pyridinium surfactants are group of the cationic gemini surfactants consisting of two positively charged hydrophilic pyridinium headgroup and two hydrophobic alkyl chains.[7] In recent years several structural derivatives of these gemini pyridinium surfactants have been developed and investigated for their physicochemical and biological properties.[8] They have demonstrated superior surface activity and ability to form complex with wide variety of organic molecules including drugs and nucleic acids.[9] They have proved to be effective antimicrobial agents as they are able to inhibit the growth and kill wide range of microorganisms.[10] One of the most prominent use of pyridinium gemini surfactants has been as non-viral gene delivery vectors as these amphiphilic molecules are able to effectively deliver DNA in wide variety of animal cells.[11] The current review article emphasizes on recent advances in the molecular design of pyridinium gemini surfactants (**Figure 1-6**), their physicochemical properties and the application area of these surfactants.

2. Structural Variety, Physicochemical Properties and Application of Gemini Pyridinium Surfactants

Several structural derivatives of pyridinium gemini surfactants containing different functional moieties have been developed in recent past. Although initial investigations demonstrated the use of the gemini pyridinium surfactants containing bipyridinium spacer for enhancing rate of electron transportation[12] and their capability to adsorb on solid surface,[13] their surfactant properties and self-aggregation behavior remained unexplored until last decade.[14] Gemini pyridinium surfactants (**1-3**) were synthesized and investigated for their self-aggregation properties by surface tension measurements.[15] The cmc values of these surfactants were lower compared to monomeric pyridinium surfactants and the values decreased with increase in hydrophobic alkyl chain length.

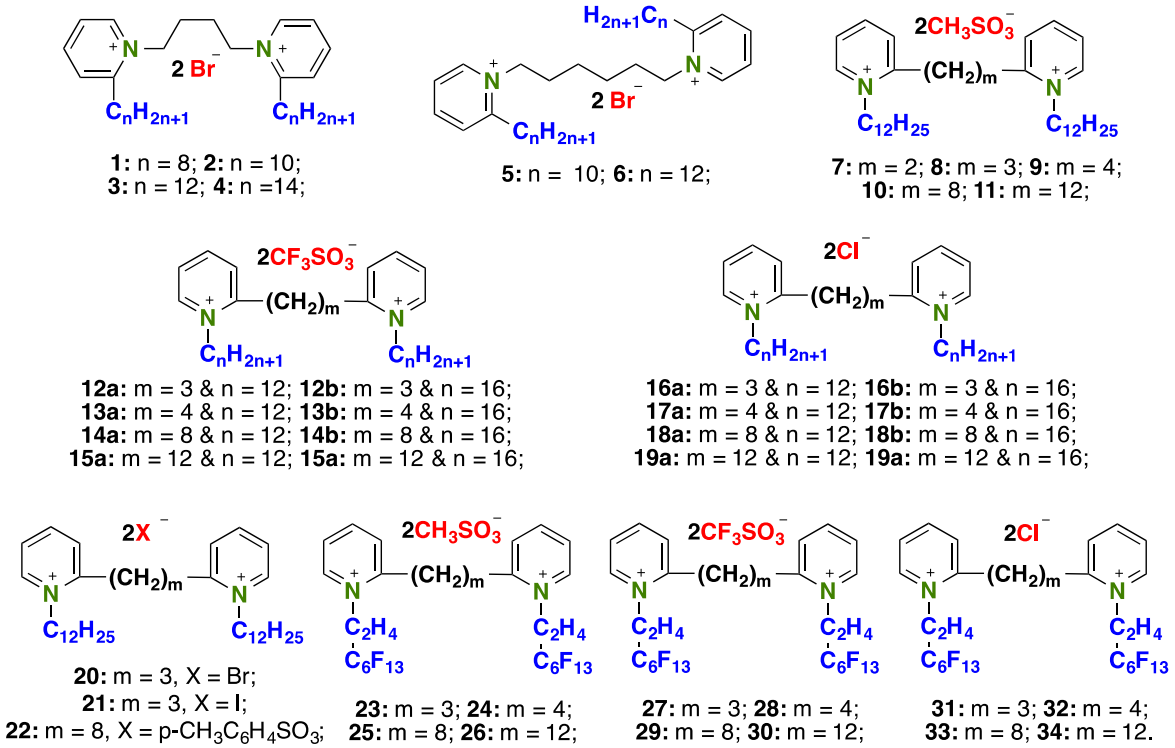


Figure 1. Molecular structure of different types of gemini pyridinium surfactants.

Gemini surfactants (**1-3**) strongly interact with polyacrylamide polymer influencing the viscosity of the mixed polymer-surfactant system and the extent of the interaction depends on the hydrophobic alkyl chain length. Gemini pyridinium surfactants (**1-6**) were investigated for their anticorrosive properties on A3 steel in 20% hydrochloric acid by gravimetric measurement and electrochemical method. The inhibitive efficiency of the gemini surfactants depends on their concentration, length of the hydrophobic alkyl chain as well as spacer length. The inhibition rate increased with increase in surfactant concentration and gemini surfactants with four methylene spacer units demonstrated better anticorrosive properties compared to gemini surfactants with six methylene spacer units. The gemini surfactants with hydrophobic decyl chains exhibited the best inhibitive efficiency. The adsorption of these gemini surfactants on the surface of A3 steel obeyed Langmuir isotherm and the positively charged pyridinium rings were able to adsorb on A3 steel surface while the hydrophobic alkyl chains formed a hydrophobic layer on the metal surface.[16]

Quagliotto *et al* synthesized several structural derivatives of the gemini pyridnium surfactants containing different hydrophobic alkyl chains, counterions and spacer units (**7-22**) and investigated these surfactants for their self-aggregation properties in aqueous solutions by surface tension and conductivity method.[14,17] These surfactants demonstrated superior surface properties compared to conventional cationic surfactants and the surface properties varied with hydrophobic alkyl chains, counterions and spacer units. Similarly, fluorinated derivatives of these gemini surfactants (**23-34**)

were synthesized and investigated for their surface properties.[18] These gemini surfactants (**23-34**) were able to reduce the surface tension of water to very low values. Partial molar enthalpies of the aqueous solutions of the gemini pyridinium surfactants (**16a**-**19a**) demonstrate a very peculiar behavior as a function of the spacer length. This behavior has been attributed to the conformation change due to the stacking interactions between the two-pyridinium rings and is presumed to have positive effect on biological applications of these gemini biological surfactant.[19] The structural analogues of these gemini surfactants (**16b**-**19b**) containing hexadecyl hydrocarbon alkyl chain were able to effectively bind and form nano complex with DNA. These gemini surfactants demonstrated good DNA delivery capability.[20]

Thioether spacer containing gemini pyridinium surfactants (**35-42**) were synthesized via regioselective co-bromination of α-olefins and 1-(allyloxy)-3-penta decylbenzene using *N*-bromosuccinimide followed by quaternization with pyridine.[21,22] These gemini surfactant have excellent surfactant properties and lower cmc values compared to conventional quaternary ammonium gemini surfactants. The surfactants (**35-42**) also demonstrated lower cytotoxicity towards animal cell lines along with excellent DNA binding capability. Gemini pyridinium surfactants (**35-38**) demonstrated hydrophobic alkyl chain length dependent DNA binding behavior and cytotoxicity trends. The complex forming ability of these surfactants with DNA increased with increase in hydrophobic alkyl chain length and the toxicity decreased with increase in hydrophobic alkyl chain length making the higher members of the series with long hydrophobic alkyl chain ideal for gene delivery applications. These surfactants effectively interact with drug phenothiazine: a tranquilizer. The mixed micellar system of the gemini surfactants and the drug were investigated in detail by several analytical and spectroscopic techniques. Analytical experiments established that the mixed micellar system of the pyridinium gemini surfactants and drug exhibited better interfacial properties than the pyridinium gemini surfactants and drug individually establishing synergistic effect between them. Further the spectroscopic techniques: fluorescence, UV-visible and NMR studies confirmed that the pyridinium gemini surfactant and phenothiazine effectively interact by the combination of both hydrophobic interactions and cationic-π interactions which influences the physico-

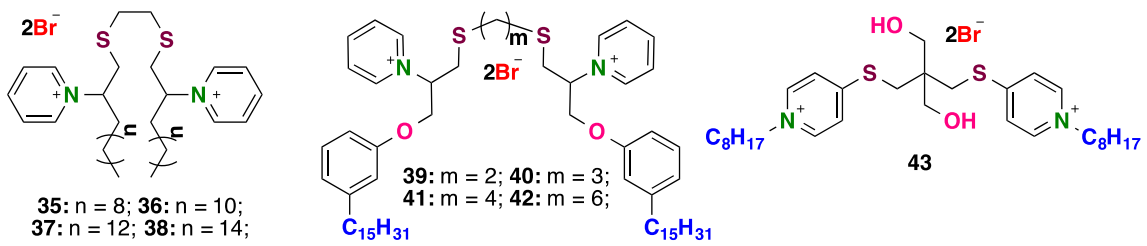


Figure 2. Molecular structure of gemini pyridinium surfactants containing thioether functionalized spacer units with/without other functional moieties.

chemical properties of the system. The studies were helpful in understanding the drug-surfactant interactions in a broader term.[23]

Similarly, the phenoxy ring containing gemini pyridinium surfactants (**39-42**) demonstrated greater ability in forming complexes with the DNA along with low toxicity towards animal cell lines. The toxicity of these surfactants was found to be better than the commercially available transfecting agent dimethyldioctadecyl ammonium bromide.[22] Thioether spacer containing gemini pyridinium surfactant (**43**) demonstrated high bactericidal potency and was used to prepare antibacterial material by immobilizing the surfactants on ferrite powder. The reaction between the hydroxyl group of the pyridinium surfactant and trimethoxysilane gave material having bactericidal potency against broad range of microorganisms.[24]

Alkyloxypyridinium gemini surfactants (**44-49**) having dodecyl, tetradecyl hydrophobic alkyl chain lengths and three different spacers were synthesized in a two step process. These gemini surfactants demonstrated excellent surface properties and have low cmc values. These surfactants were able to protect the DNA against enzymatic degradation and were found to be less cytotoxic compared to conventional quaternary ammonium gemini surfactant. The DNA compaction and protection ability combined with low toxicity make these molecules ideal for gene delivery application.[25] The size of the micelles formed by these gemini surfactants in aqueous solution decreased with increasing in spacer length.

Gemini pyridinium surfactants (**50-53**) with hydroxyl group containing spacer and different hydrophobic alkyl chain lengths were synthesized in a two-step process. The presence of an ether and a hydroxyl group close to the pyridinium headgroup have marked influence on the physico- chemical properties of there surfactants as the micellization process in influenced by the hydrogen bonding between the surfactant molecules and water present in the bulk. The DLS and TEM experiments established that the size of the micellar aggre-

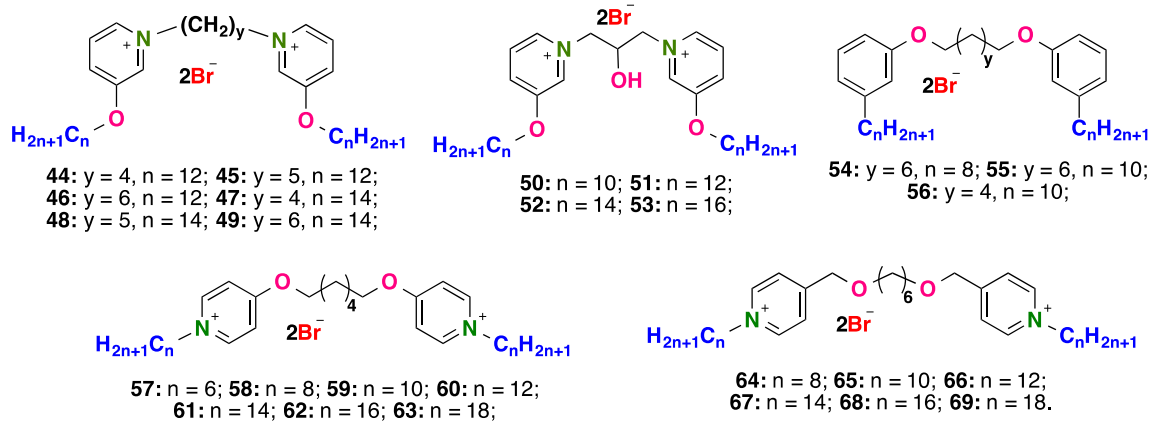
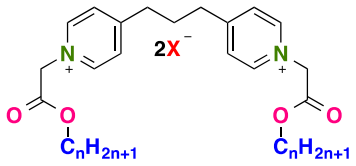


Figure 3. Molecular structure of gemini pyridinium surfactants containing ether functional group with/without other functional moiety.

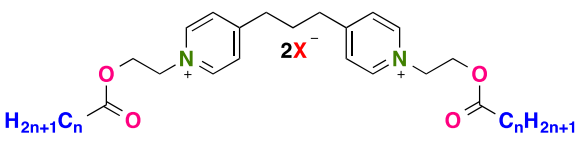
gates depends on the hydrophobic alkyl chain length and it increases with increase in the hydrophobic alkyl chain length. These surfactants also demonstrated hydrophobic alkyl chain length dependent DNA compaction ability which was established by EB exclusion experiments, gel electrophoresis and zeta potential measurements. The complex formed by these gemini surfactants with DNA were stable against enzymatic degradation. The cytotoxicity of these surfactants were investigated on living cell lines: BV2 and C6 glioma. The presence of polar hydroxyl group also influences the cytotoxicity trends of these surfactants as they were found to be less toxic compared to conventional quaternary ammonium gemini surfactants.[26]

Gemini pyridinium surfactants (**54-69**) demonstrated high activity against wide variety of microbes and were able to inhibit the growth of microorganisms at very low concentration. These surfactants were effective in killing majority of Gram-positive bacteria (i.e. *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, and *Micrococcus luteus*). They were also found effective against Gram-negative bacteria (i.e. *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus rettgeri*, *Proteus mirabilis*, *Escherichia coli* and Salmonella *enteritidis*). They were also found to be effective against several varieties of fungus, yeast and molds (i.e. *Candida albicans*, *Candida utilis*, *Torulopsis xylinus* and *Saccharomyces cervisiae*, *Aspergillus niger*, *Aureobasidium pullulans*, *Penicillium funiculosum*, *Gliocladium virens*, *Chaetomiun globosum*, *Rhyzopus oryzae*, *Rhyzopus stronifer*, *Trichophyton rubrum* and *Mycotecium verrucaria*).[27,28]

Ester based gemini pyridinium surfactants (**70-75**) having different hydrophobic alkyl chain length and different counterions (i. e. Cl and Br) were synthesized in a two step process starting from linear fatty alcohols. These surfactants demonstrated excellent surface properties and the cmc values of the surfactants decreases with the increase in hydrophobic alkyl chain length and size of the counterion.[29] Further, reverse micelles of these gemini pyridinium surfactants encapsulate and remove methyl orange dye from aqueous phase in amyl alcohol solvent. The encapsulation and removal of the dye depends on the size of the reverse micelle of the surfactant. The size of the counterions also influences the dye recovery as the gemini surfactants with bromide counterions demonstrated greater ability to remove dye compared to their chloride analogue. The efficiency to



70: n = 12, X = Cl; **71:** n = 14, X = Cl; **72:** n = 16, X = Cl;
73: n = 12, X = Br; **74**: n = 14, X = Br; **75**: n = 16, X = Br;



76: n = 11, X = Cl; **77:** n = 13, X = Cl; **78:** n = 15, X = Cl;
79: n = 11, X = Br; **80**: n = 13, X = Br; **81**: n = 15, X = Br.

Figure 4: Molecular structure of gemini pyridinium surfactants containing ester functional group.

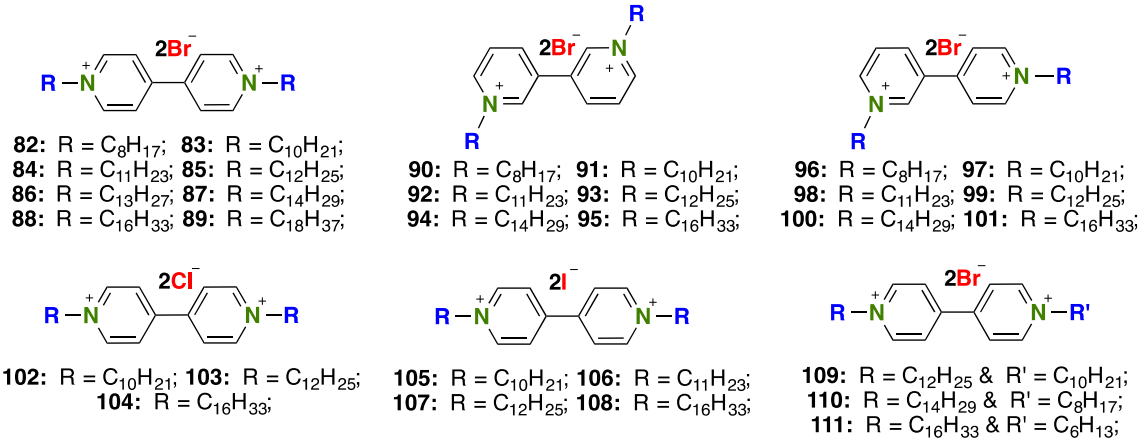


Figure 5: Molecular structure of gemini pyridinium surfactants containing bipyridinium moiety as headgroup as well as spacer.

remove dye molecule linearly increases with the increase in surfactant concentration as at higher concentration more number of reverse micelles are formed in the solvent.[30] Similarly gemini pyridinium surfactants (**76-81**) synthesized in a two step process demonstrated hydrophobic alkyl chain length dependent surface properties and thermal stability.[31]

Gemini pyridinium surfactants (**82**-**111**) having different bipyridinium moieties were found to be very effective antibacterial agent against wide range of Gram-positive and Gram-negative bacteria. The antimicrobial activity of these compounds depends on the nature of bipyridinium moieties, hydrophobic alkyl chain length and counterions associated with the surfactant molecules. They were found to be effective against Gram-positive (*Staphylococcus aureus* and *Enterococcus faecalis*) and Gram-negative (*Pseudomonas aeruginosa* and *E. coli*) strains.[32,33] Further the antimicrobial activity of these gemini derivatives were compared with their monomeric counterparts and in most of the cases the gemini surfactants were found to be much more effective compared to monomeric analogues. Most of these gemini surfactants were fourfold effective against the bacteria compared to commercially available cationic surfactants: benzalkonium chlorides. Some of these gemini derivatives were also found to be effective against QAC resistance and methicillin-resistant *Staphylococcus aureus* (MRSA).[34] Gemini pyridinium surfactants (**82**, **83**, **85**) acted as phase transfer catalyst and catalyzed Wolff-Kisner and Huang Ming-Long reductions.[35] The results also show that these gemini surfactants were more effective in deoxidizing bis-alkyl diphenyl ketone compared to commercially available cationic surfactant CTAB. Gemini pyridinium surfactant (**89**) was also able to form interfacial assemblies with cyanic dyes.[36] Gemini pyridinium surfactants (**112**-**123**) were synthesized and tested for gene delivery application. These gemini pyridinium surfactants (**112-118**) demonstrated spacer length dependent transfection efficiency as their gene delivery capability varied with the length of spacer units.

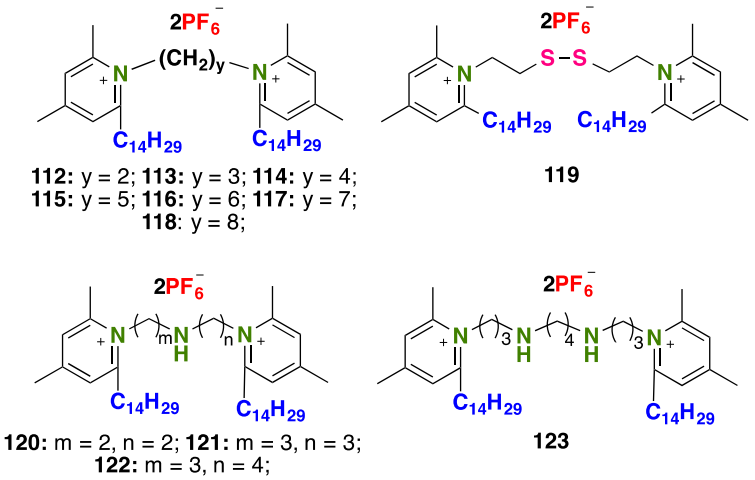


Figure 6: Molecular structure of gemini pyridinium surfactants designed for gene delivery application.

Increasing the methylene spacer units from two to three drastically decreases the transfection efficiency of the gemini surfactants. Further elongation to four and five spacer units improved the transfection efficiency. The higher members of the series demonstrated higher transfection efficiency as the gene delivery capability significantly improved for gemini surfactants with six and seven spacer units. However the last member of the series gemini surfactant (**118**) exhibited lowest transfection efficiency among the series. Gemini pyridinium surfactant (**119**) having disulfides was also investigated for its gene delivery capability however this surfactant exhibited high cytotoxic effects on NCI-H23 cell and was found to be totally ineffective for gene delivery application. Gemini pyridinium surfactants (**120**-**123**) were also found to be less effective gene delivery agents compared to their structural analogues (**112**-**118**).

3. Summary

Gemini pyridinium surfactants have demonstrated superior physicochemical properties and are currently being investigated for several application areas. They are able to self-aggregate in aqueous solution at very low concentration and are able to influence solution properties of the aqueous system. Several structural derivatives of these surfactants have been developed in recent years having unique properties. They have unique ability to condense and form complex with DNA and have demonstrated their effectiveness as non-viral gene delivery agents. They are also able to form complex with drug and can be used as drug delivery agents. Beside their probable use as gene and drug delivery agents, they have also demonstrated their effectiveness as antimicrobial agents since they are able to retard the growth and kill several disease causing microorganisms. In many cases they have been found to be even better than most of the commercially available cationic surfactants. They are able to adsorb on metal surfaces and act as anticorrosive agent. They also show unique

capability to form complex with a wide range of protein molecules. The continued effort to develop and investigate several new structural motifs of the gemini surfactants led to the development of many new types of gemini pyridinium surfactants. These surfactants have demonstrated their effectiveness for several application areas. We have tried to summarize different structural variety of the gemini pyridinium surfactants along with their properties in the current review article.

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