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Bio-inspired surfactants capable of generating plant volatiles†

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Plants are able to synthesize, store and release lipophilic organic molecules known as plant volatiles (PVs) utilizing specific biological pathways and different enzymes which play vital roles in the plant's defence and in dealing with biotic and abiotic stress situations. The process of generation, storage and release of PVs by plants acquired during the course of evolution is a very complex phenomenon. Bio-inspired molecular design of farnesol-based surfactants facilitates similar production, storage and release of PVs. The designed molecules adsorb at air–water interface and self-aggregate into micelles in aqueous system. The structural design of the molecules allows them to self-activate in water *via* intramolecular cation-π interactions. The activated molecules undergo molecular rearrangements generating volatile organic molecules both at interface and inside the micelle core. The molecules adsorbed at the interface initially release the formed volatile molecules creating vacant space at interface, thus thermodynamically directing the micelle to release the manufactured volatile products.

Introduction

Nature has always inspired mankind to develop future technologies based on its simple but efficient working pattern and many designs in nature are often based on the concept of self-assembly.[1] The process of evolution has enriched living systems with incomparable levels of design, which is maintained by the encoded information in their genetic material. Plants are unique in this regard. Despite the extremely limited locomotion capability they have the ability to control their surroundings by releasing important PVs, which influence the behavior of other living organism nearby.[2] Plants are able to synthesize, store and release PVs which play vital roles in their defense, tritrophic interactions, plant–plant communication and in dealing with biotic and abiotic stress situations.[3] When attacked by herbivores they release specific herbivore-induced PVs[4] or characteristic terpenoids to attract carnivorous enemies of the herbivores.[5,6] Plant can be chemically provoked to release PVs for defense.[7] Furthermore, genetically modified plants capable of releasing increased levels of herbivore-induced volatiles demonstrate a greater ability to attract carnivorous predators, thereby increasing the plant defenses.[8,9] The long distance signaling among plants with the help of PVs is still a well debated topic of research.[10–12] Volatile isoprenoids provide protection to plants against several abiotic stresses including light, temperature, drought and oxidizing conditions of the atmosphere.[13] Among the various PVs generated and emitted by the plants the bulk of them belong to the terpene family and almost all plants have the potential to manufacture terpenes for certain essential physiological functions.[14] However, the importance and function of the numerous terpenes remains underexplored and investigations are lagging behind, especially to understand the chemistry of plant volatiles, and their practical application in medicine, agriculture and industry. To best of our knowledge, no chemical systems have been discovered that mimic the *in situ* synthesis, storage and release of PVs. Since PVs are important signaling molecules, the deciphering of the chemical signals may be useful for designing new sustainable methods for pest and environmental control.[15]

In recent years the area of surfactant science has witnessed robust progress and researchers have been successful in designing several category of stimuli responsive surfactants which response to change in pH, temperature, CO_2, light and magnetic field.[16] Here-in, unique set of new surfactants capable of generating and releasing variety of volatile organic molecules in aqueous system has been developed. We in the past have designed several biobased/biocompatible molecules/materials taking clue from the working pattern in nature.[17–19] In continuation of our work we have designed series of farnesol-based heterocyclic cationic surfactants capable of self-activating themselves when dissolved in water *via* intramolecular cation-π interactions. The activated molecules undergo hydrolysis and rearrangements generating and releasing volatile organic

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molecules as displayed in nature by plants. The mechanistic strategy adopted to stimulate these molecules in aqueous system consists of activating the double bond present at β position of farnesyl chain adjacent to ester functional group of designed molecule by a heterocyclic cationic system present within the molecule, thus directing them to generate reactive intermediate species capable of undergoing rearrangements generating PVs.

Results and discussion

The farnesyl pyrophosphate (diphosphate) is the precursor for the production of large array of PVs in the plants. The diphosphate dissociation from the enzyme-bound acyclic farnesyl diphosphate generates an allylic carbocation that electrophilically attacks double bond of terpene chain which further undergo rearrangements producing several cyclic and acyclic sesquiterpenes in plants.[20]

Farnesyl diphosphate can be considered an amphiphilic molecule containing both hydrophobic and hydrophilic moieties within same molecule (Fig. 1). Taking cue from this naturally occurring molecule we designed a series of amphiphilic molecules (Scheme 1) capable of self-activating, undergoing rearrangements, producing and subsequently releasing volatile organic molecules.

These amphiphilic molecules typically behave as surfactants when dissolved in water as they diffuse to the air–water interface and reduce the surface tension of the water. The migration process of the surfactants to the air–water interface and the corresponding decrease in surface tension value of water continues until the air–water interface becomes fully occupied by surfactant molecules and no vacant space is available at the interface.[21] At this point the farnesol-based surfactants begin to form micelle in an aqueous system and there is no further decrease in surface tension value. Farnesol based cationic surfactants are able to form micelles at this point is known as the critical micelle concentration (cmc).

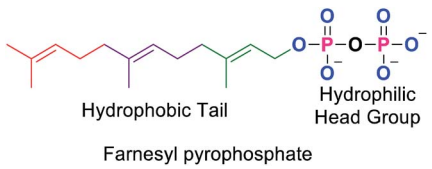


Fig. 1 Structure of farnesyl pyrophosphate. The molecule contains both hydrophobic carbon chain (farnesyl moiety) and a hydrophilic head group (diphosphate moiety).

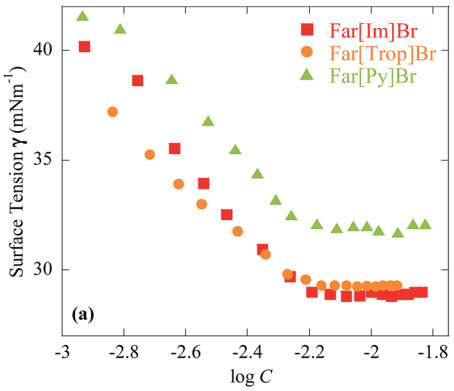
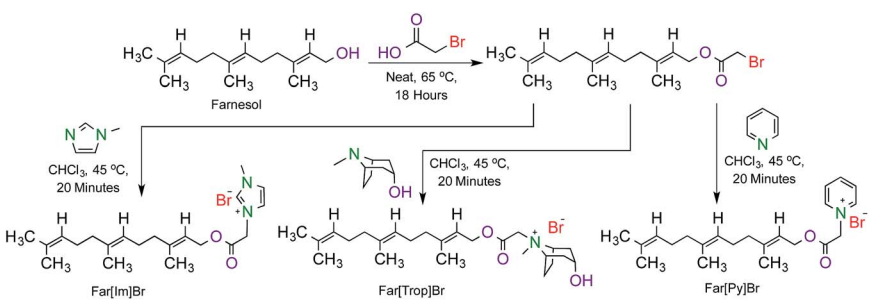


Fig. 2 Surface tension *vs.* log *C* plot of the farnesol-based surfactants.

Fig. 2 shows the plot of surface tension *versus* log of concentration for farnesol based cationic surfactants. The break point in the graph corresponds to the cmc value of individual surfactant. Far[Py]Br was able to form micelles at 5.90 mM concentration and have the lowest cmc value among the series of surfactants investigated, however Far[Im]Br has been found to reduce the surface tension of water to a greater extent when compared to other farnesol based surfactants. The cmc values of these surfactants have also been investigated by conductivity method.[22,23] The results of conductivity experiments further confirmed the ability of farnesol based cationic surfactants to form micelles in aqueous solution (Fig. 3). Physical parameters of farnesol-based surfactants have been calculated from the data obtained from surface tension and conductivity experiments (Table 1).

The calculated free energy of micellization (ΔG_{mic}°) of these farnesol based cationic surfactants has been found to be negative which suggests that micellization is a thermodynamically favourable and spontaneous process for these molecules when all the interface is occupied by surfactant monomers.[24]

The degree of counterion binding (β%) shows the bromide counterions present in the stern layer of micelle to counterbalance the electrostatic force which opposes micelle formation.[25] The β value indicates the ability of counterion to bind



Scheme 1 Synthesis of farnesol-based surfactants.

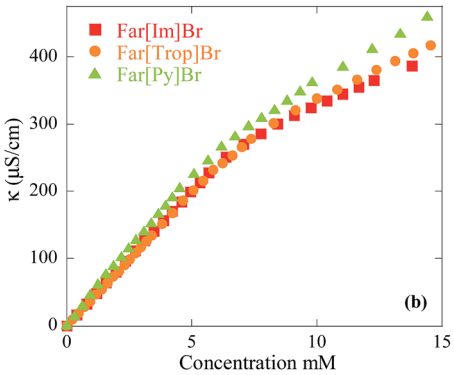


Fig. 3 Specific conductivity *vs.* concentration plot of farnesol-based surfactants.

micelles which has been found to be maximum for Far[Im]Br. The experimental results of the self-aggregation studies of farnesol-based surfactants revealed that the nature of hydrophilic head group greatly influences the aggregation behavior of each individual surfactant in aqueous system.

The aqueous solutions of farnesol-based surfactants were further investigated above their cmc values by time dependent ^{1}H NMR spectroscopy. The results provided further insight into solution chemistry of these molecules. The activated farnesol based surfactants undergo time dependent isomerization and hydrolysis in the aqueous solution. ^{1}H NMR studies further established that different surfactants isomerize and hydrolyze at different rates and the process is dependent upon the nature of the hydrophobic head group attached to the farnesyl moiety. Far[Im]Br starts to hydrolyze after 36 hours and completely hydrolyzes in 144 hours (Fig. S1†). By contrast, the pyridinium analogue (Far[Py]Br) undergoes very fast isomerization and hydrolysis as it start to hydrolyze after 6 hours and is completely hydrolyzed by 48 hours (Fig. 4). Far[Trop]Br starts to isomerize and hydrolyze by 18 hours (Fig. S2†) and the process is completed in 110 hours.

Surface studies established that these farnesol based surfactants self-aggregate to form micelles at different concentrations depending upon the nature of the cationic hydrophilic head present in the molecule. The calculated surface parameters and thermodynamic parameters also differ for different surfactants under investigation. Correspondingly different surfactants have different levels of self-activation when dissolved in water. Interestingly, the first-hand macroscopic

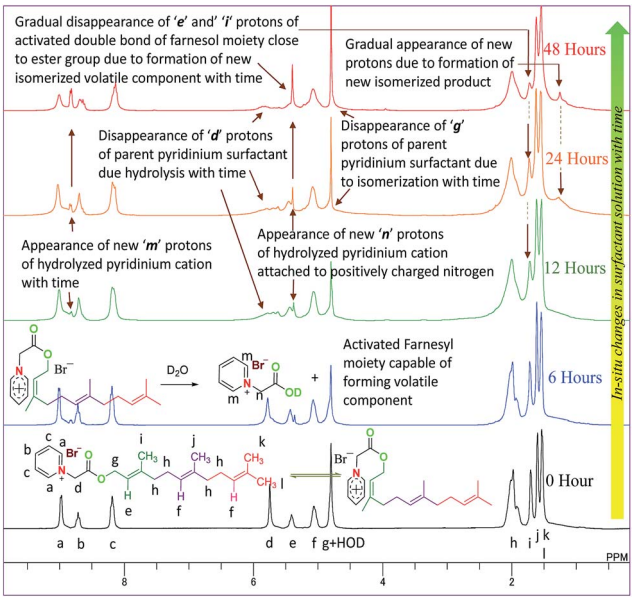


Fig. 4 Time dependent ^{1}H NMR studies of 50 mM solution of Far[Py]Br in D_2O. The NMR spectra shown at different time interval: 0 hour, after 6 hours, 12 hours, 24 hours and 48 hours respectively at 25 °C. The surfactants solution were kept at 25 °C. The surfactant starts to degrade after 6 hours and completely degrades in 48 hours (degradation time determined by recording NMR on short time interval).

observation was that we noticed the odorless surfactant assemblies generate mild pleasant smell (fragrance-like) after certain time in water at ambient conditions. Further, the changes evident from the time dependent ^{1}H NMR spectroscopy encouraged us to investigate the solution chemistry in detail by headspace GC-MS analysis by analyzing the type of volatile components being generated in the aqueous solution inside the micelle core. Since different surfactants undergo isomerization and hydrolysis at different rates, each individual system was investigated at different time intervals depending on the information available by time dependent ^{1}H NMR spectroscopy.

The initial analysis of air samples withdrawn from the headspace of each individual surfactant solution shows 5 main peaks. These peaks corresponds to (*Z*)-nerolidol, (*E*)-nerolidol, two of their activated derivatives, and an undetermined peak by hydrolyzed heterocyclic ionic liquid derivative. The two isomers of nerolidol are formed by rearrangement and hydrolysis at the interface of the aqueous system. However, when the samples

Table 1 Surface properties of farnesol based cationic surfactant at 25 °C

Surfactant	cmc^a (mM)	cmc^b (mM)	β%	γ_{cmc} (mN m^{-1})	$10^6\ \Gamma_{max}$ (mol m^{-2})	A_{min} (nm^2)	ΔG_{mic}° (kJ mol^{-1})	ΔG_{ads}° (kJ mol^{-1})
Far[Im]Br	6.77	6.85	60	28.8	1.47	1.13	−35.7	−65.1
Far[Trop]Br	6.20	6.79	55	29.2	1.38	1.20	−34.6	−65.6
Far[Py]Br	5.90	5.96	48	31.9	1.35	1.23	−33.5	−63.2

^{a} Determined by pendent drop method. ^{b} Determined by conductivity method.

were analyzed for the volatile components after it had undergone complete hydrolysis, several different fractions of PVs were detected (Fig. 5). The initial results of headspace analysis of the samples seem quite obvious because during the initial stages when the surfactants are dissolved in the water, the molecules tend to migrate to the air–water interface due to their surfactant nature and subsequently when the interface is completely occupied the surfactant monomers start to form micelles. The molecules present at the air–water interface experience a different chemical environment compared to molecules that are part of the micelle. The hydrophobic farnesyl chain of the surfactant molecules at the interface is slightly exposed to an aqueous environment, while those parts of the micelles that remain inside the hydrophobic micellar core are parts of the hydrophobic environments. The results of headspace sample analysis confirmed that initial changes occurring at the air–water interface rather than in the bulk solution. Two probable mechanisms can be conceived for the synthesis of PVs starting from farnesol based cationic surfactants. One occurs at the interface in an aqueous environment and the other occurs inside the hydrophobic environment in the micelle core.

Isomerization and hydrolysis of monomeric molecules at the interface generate volatile organic molecule and an ionic liquid. However, the activated surfactant monomers, which are the part of the micelle, continue to generate and store PVs inside the micelle core and the consistency and structure of micelle structure is preserved because all molecules do not undergo structural changes at the same time (as observed by ^{1}H NMR spectroscopy).

The changes occurring at the interface create vacant empty spaces at the air–water interface. Since free energy of adsorption (ΔG_{ads}°) is always greater (more negative) than the free energy of micellization (ΔG_{mic}°), which is also true for the farnesol-based surfactants under investigation (Table 1), the micelles break down releasing the manufactured organic volatile as well as surfactant monomers in the aqueous solution. The released surfactant monomers migrate to the air–water interface to occupy the vacant space created by the hydrolyzing and isomerizing surfactant molecules at the interface. The generated organic molecule remains in water while some escapes into the air. The calculated thermodynamic parameters support this hypothesis. This process continues until the entire micelle is consumed generating the volatile organic molecules. A gradual change in turbidity of the aqueous solution can be observed over time, as most of the volatile organic molecules formed are practically insoluble in water, although they remain as an emulsion due to the emulsifying nature of surfactants present in the solution.

During the changes occurring at the air–water interface and inside the micelle core the hydrophilic head group dissociates from the parent surfactant molecule to form heterocyclic ionic liquids in the solution. Most of the generated ionic liquids move out to the aqueous solution as head groups are present at periphery of micelle, however some may be able to penetrate into the micelle core and catalyze the formation of different types of products. This assumption is based on the volatile molecules detected from the headspace GC-MS (Fig. 6). The major volatile organic molecules generated inside the micelle core are: (*Z*)-β-farnesene, (*E*)-β-farnesene, (*Z*,*E*)-α-farnesene, (*E*,*E*)-α-farnesene, (*E*,*Z*)-α-farnesene, (*Z*,*Z*)-α-farnesene, β-bisabolene, α-bisabolene, α-bisabolol, (*E*)-nerolidol, (*Z*)-nerolidol, isopulegol acetate and α-bergamotene. However the generated

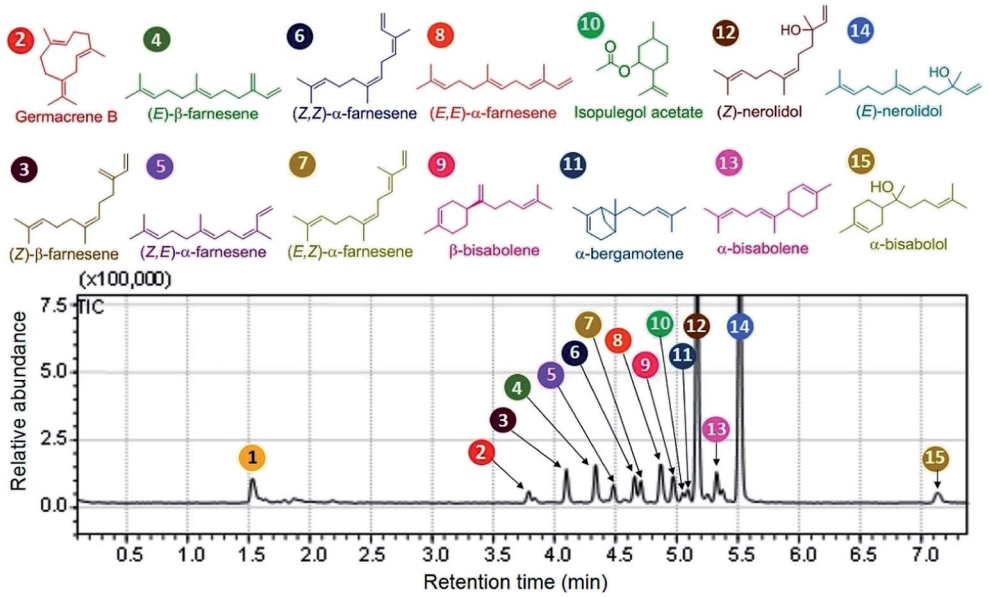


Fig. 5 Headspace analysis of 50 mM aqueous solution of Far[Trop]Br. Headspace was collected after 110 h. The main components were identified by mass spectra of computerized libraries. The chemical structure of major molecules generated inside the micelle core are 2: germacrene B, 3: (*Z*)-β-farnesene, 4: (*E*)-β-farnesene, 5: (*Z*,*E*)-α-farnesene, 6: (*Z*,*Z*)-α-farnesene, 7: (*E*,*Z*)-α-farnesene, 8: (*E*,*E*)-α-farnesene, 9: β-bisabolene, 10: α-bergamotene, 11: isopulegol acetate, 12: (*Z*)-nerolidol, 13: β-bisabolene, 14: (*E*)-nerolidol, 15: α-bisabolol. Peak 1 corresponds to unidentified hydrolyzed tropine based ionic liquid derivative.

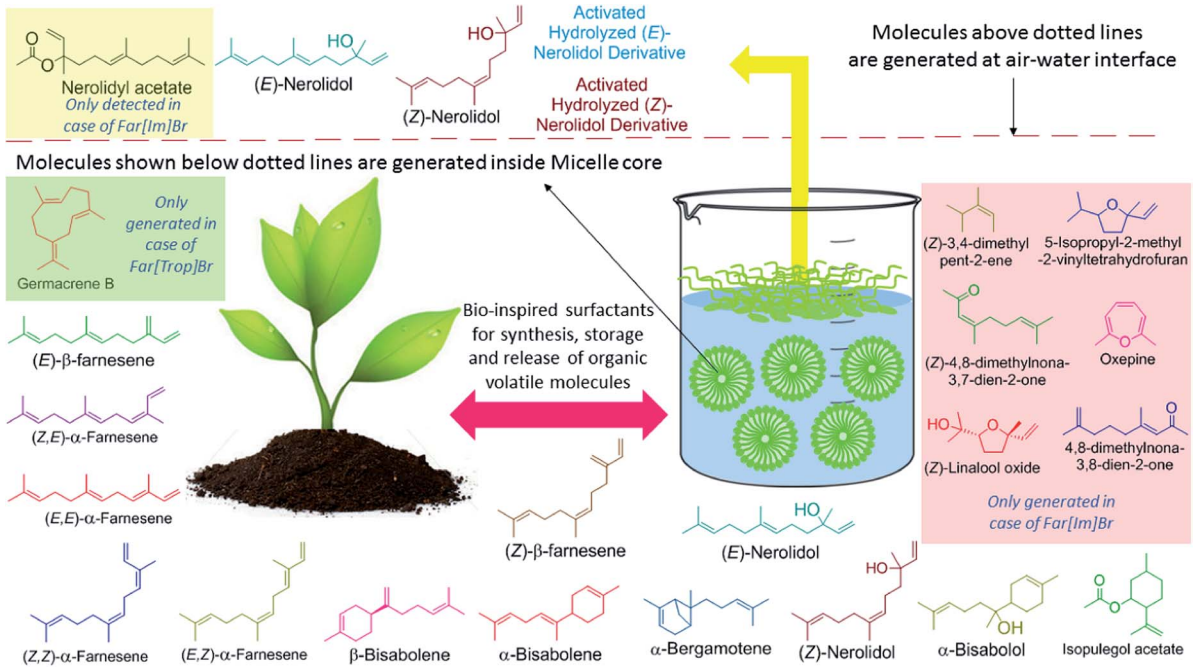


Fig. 6 Volatile organic molecules generated at the air–water interface and inside the micelle core in aqueous solution as determined by headspace GC-MS analysis.

imidazolium ionic liquids in case of Far[Im]Br may be able to catalyse formation of other organic volatiles *i.e.* 5-isopropyl-2-methyl-2-vinyltetrahydrofuran, oxepine, (*Z*)-3,4-dimethylpent-2-ene, (*Z*)-4,8-dimethylnona-3,7-dien-2-one, 4,8-dimethylnona-3,8-dien-2-one and (*Z*)-linalool oxide (Fig. S3†), which were not detected in case of other structural analogues. Similarly, the dissociated tropine based ionic liquids are able to catalyse the formation of germacrene B in the case of Far[Trop]Br (Fig. S4†) not detected in case of Far[Im]Br and Far[Py]Br. Currently we are investigating the role of dissociated ionic liquids for their role as catalyst in the formation of different volatile component.

The structural design of the farnesol based heterocyclic cationic surfactants enables them to self-activate in aqueous solution by forming cation-π complex. The double bond at the β position of the farnesyl moiety adjacent to ester functional group interacts with the positive charge on the hydrophilic cationic head group of the surfactant molecule. The activated molecules are able to isomerize and form different type of volatile organic molecules.

To understand the interaction of farnesol-based surfactants in the aqueous system we synthesized a reference citronellol based cationic amphiphile (ESI†) and investigated both farnesol and citronellol based amphiphile by NMR spectroscopy using D_2O as solvent. The –NC*H*N– proton of imidazolium cation is strongly deshielded. Imidazolium cation across the front (*i.e.* –N–C^2–N–) can be described by delocalized three center $4e^-$ component. The hydrogen at C^2 position is more acidic because imidazolium cation forms C=N π bond, leaving the C^2 carbon with concentrated positive charge and electron deficient (Fig. 7).[26]

The hydrogen attached to C^2 carbon of imidazolium cation in citronellol-based amphiphile is strongly acidic and undergoes exchange with deuterium when dissolved in D_2O (Fig. 7a). In contrast, the hydrogen attached to C^2 carbon of imidazolium cation in the case of Far[Im]Br does not undergo exchange with deuterium. It is evidenced that in case of citronellol based amphiphile, formation of cation-π complex does not occur, while it do occur in case of farnesol-based surfactant leading to compensation of the concentrated positive charge on the C^2 carbon of the imidazolium cation thereby making the

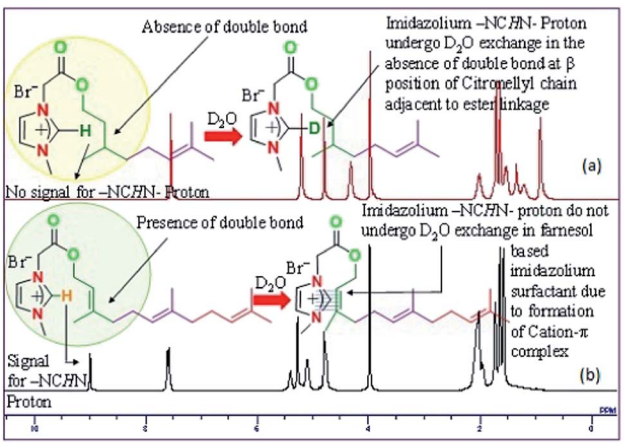


Fig. 7 ^{1}H NMR spectra of (a) citronellol based amphiphile and (b) farnesol based cationic surfactant in D_2O. The imidazolium –NC*H*N– proton do not undergo D_2O exchange in case of (b) due to presence of cation-π interactions.

proton attached to it less acidic. This structural feature is prevalent in aqueous system when both a cation and a π system are present in close proximity.[27] A closely related system to our designed cation-π system is the indole-3-acetic acid choline ester model investigated by Aoki *et al.*[28]

An important factor regulating the rate at which the volatile organic molecules are formed is controlled by the extent of the cation-π interaction.[29] Each individual heterocyclic moiety have different magnitude of the cation-π interactions. The imidazolium cation interacts with π-electrons of the double bond β to the farnesyl ester group through a delocalized positive charge distributed across the –N–C^2–N– front region, while the pyridinium cation probably interacts with the delocalized positive charge on its ring with a larger sphere of positive charge and tropine with a positive charge centered on the quaternary nitrogen atom. However, apart from activation through formation of a cation-π complex structural arrangement of molecules also plays an important role. Far[Im]Br is able to form tighter packing at the air–water interface as evident from the calculated A_{min} value (the area per molecule at the interface), this ability decreases in the order as Far[Im]Br > Far[Trop]Br > Far[Py]Br. Consequently, Far[Im]Br undergoes a slow isomerization and hydrolysis compared to the others which have higher A_{min} values. Since a tighter packing at the interface restricts the movement of molecules principally involved in rearrangement, Far[Im]Br undergoes a slow isomerization and hydrolysis at the interface compared to others.

Conclusion

Bio-inspired farnesol-based surfactants capable of self-activating and self-aggregating in aqueous system have been designed. These surfactants are able to adsorb at air–water interface and self-assemble as micelles in aqueous solution. The micelle core acts as center for generation and storage of volatile organic molecules where the activated aggregated molecules rearrange to form several organic volatile molecules. The molecules at the air–water interface undergo rearrangement and hydrolysis creating new vacant space at interface thus thermodynamically directing the micelle to release surfactant molecules as well as the manufactured products. The rate of generation of volatile organic molecules formed inside the micelle core as well as the types of the volatile molecule formed can be controlled by varying the type of heterocyclic cationic moiety attached to the parent farnesyl chain. Since each individual cationic system activates and function in a specific manner it is possible to control the rate at which the volatiles are generated. During the physicochemical changes taking effect in the aqueous solution the detaching/hydrolyzing cationic head group forms heterocyclic ionic liquids that further catalyze the formation of different organic volatiles inside the micelle core in each individual system under investigation.

The current research findings open new door in the field of surfactant science and in investigating the chemistry of PVs. In nature, plants are able to generate, store and release volatile organic molecules including many types of volatile fragrances. Several new molecules can be designed capable of generating, storing and controlled releasing fragrance molecules. Such new fragrance release technology combined with detergency can be utilized in both consumer and industrial applications.

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