BIOCOMPATIBLE CHOLINIUM FATTY ACID-BASED IONIC LIQUIDS AS GREEN ALTERNATIVES TO CONVENTIONAL SURFACTANTS FOR BIOMEDICAL APPLICATION

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九州大学

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BY

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In the name of Allah, The Most Beneficent and The Most Merciful

This thesis is fully dedicated to my beloved parents and family

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ABSTRACT

Developing a universal drug delivery vehicle of sparingly soluble drugs remains a challenge because of their limited solubility, permeability and bioavailability. Recently, microemulsions (MEs) have attracted significant interest as promising smart drug delivery carriers for topical and transdermal drug delivery (TDD) owing to their straightforward preparation, long-term stability, biocompatibility and high drug solubilization capacity for potential biomedical applications. In the last decade, ionic liquid (ILs)-based MEs have been tested as potential nanocarriers for TDD. Surface-active ionic liquid (SAIL)-based ionic liquid-in-oil (IL/O) microemulsions (MEs) are considered as the most suitable vehicles due to their better surface-active properties, enhanced antimicrobial and skin permeation activity, high temperature stability and temperature insensitivity, and even they considered as environmentally friendly surfactants as compared to conventional surfactants. However, most of the SAILs are not suitable for potential applications because toxic cations such as ammonium, imidazolium, pyrrolidinium and morpholinium are used. To address these issues, eco-friendly and biodegradable cholinium fatty acid or amino acids containing SAIL-based MEs are required, where the SAILs are potential alternatives to conventional surfactants for envisaging biomedical applications.

Here we synthesized a series of halogen-free and biocompatible choline–fatty-acid-based ILs with different chain lengths and degrees of saturation, and we then investigated their micellar properties in aqueous solutions. The critical micelle concentration of the SAILs was found to be 2 to 4 times lower than those of conventional surfactants. The thermodynamic properties of micellization (ΔG^0_m, ΔH^0_m, and ΔS^0_m) indicated that the micellization process of the SAILs is spontaneous, exothermic, and entropy-driven at room temperature. Interestingly, the size of spherical micelles was within a range of 3.9-5.6 nm in diameter. Moreover, the toxicity profile indicates that the SAILs are practically harmless and less toxic than that of conventional surfactants. Then the SAILs were formulated to prepare novel IL/O MEs composed of a SAIL, sorbitan laurate (Span-20) and isopropyl myristate. Importantly, dynamic light scattering (DLS) analysis of the prepared MEs with or without drugs clearly demonstrated excellent physico-chemical stability of the MEs and the presence of spherical micelle formation with diameters ranging from 6.5 to 21.2 nm. Regarding the cell viability studies, the SAIL-based MEs (94%) showed a significant alternative of conventional surfactant-based MEs. The significant solubility enhancement of these drugs using this SAIL-based MEs is most likely because of the formation of hydrogen bonds and electrostatic interactions among the head groups of SAIL surfactants, the anions of ILs and the polar groups of drug molecules. The obtained results suggest that SAILs surfactants could be a potent biocompatible alternative to conventional surfactants and the introduced SAIL-based MEs represent promising target drug delivery systems.

CHAPTER 1: IONIC LIQUIDS BASED DRUG DELIVERY SYSTEMS: CHALLENGES AND OPPORTUNITIES FOR PHARMACEUTICAL INDUSTRY

1.1. Introduction

The progress in efficient drug delivery systems (DDS) for numerous disease management has been a problematic task for pharmacological scientists without negotiating on safety and efficacy of drugs. Pharmaceutical scientists are searching the new formulations or improving the current formulations of drugs so that the unmet medical behavior of drugs could be discovered. Novelty of new drugs or dosage forms and alteration of existing form of drugs or formulations causes to significantly increase the number of drugs which possess poor biopharmaceutical properties. [1–3] Moreover, pharmaceutical industries have been trying to take profitable tactics for filing in NDA under 505(b)(2) with faster dissolution and enhanced bioavailability by developing or improving the existing formulations of these insoluble drugs.[4] In 2019, the Center for Drug Evaluation and Research of FDA's accepted 59 novel drugs where 42 New Chemical Entities and 17 Biologics drugs, which is the highest in last few decades (Figure 1.1).[5]

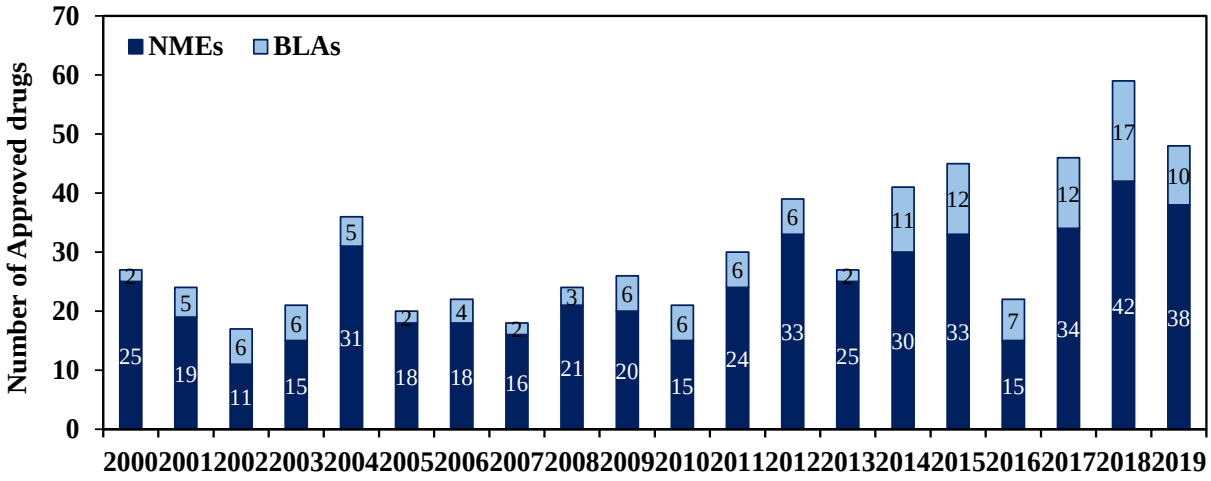

Figure 1.1. FDA approvals novel drugs since 1993. CDER approved annual numbers of new molecular entities (NMEs) and biologics license applications (BLAs). Figure reproduced with permission from ref.[5]

Nearly 40% of the top 200 oral drugs which are marketed in the US and Europe, almost 90% of the developed pipeline drugs, 75% of compounds under development and 33% of FDA drugs have been suggested to poorly soluble compounds.[3,6,7] These marketed drugs are showed poor solubility, low permeability, rapid metabolism and elimination from the body as well as poor

safety and tolerability.[4,8,9] Thus, it is essential to understand the problematic characteristics and properties of drug molecules to develop the effective formulations of these marketed drugs.

1.2. Problems of conventional drugs

1.2.1. Low solubility and bioavailability

The drug therapeutic efficacy generally depends on its bioavailability, which directly interrelated to the drug solubility and permeability.[10] An orally administered drug signifies the lower dissolution in body liquids with low absorption via the gastrointestinal tract (GIT) because of the limited aqueous solubility.[9,11] Usually, when the drugs dissolve lower than 100 μg/mL in aqueous or biological fluids such as PBS, SIF, or SFG are known as dissolution-limited absorption.[2,12] In these cases, limited permeability which is barriers to drug delivery, low therapeutic windows and systemic side effects with a limited short half-life is observed in their respective clinical applications.[9,11] Consequently, topical toxicity in the GIT upon oral administration is occurred due to higher doses of drugs for reaching the therapeutic effect which causes.[2] In addition, formulation development for higher doses is generally difficult due to their poor powder flow ability and higher sticking tendency during granulation and tableting process.[2]

1.2.2. Polymorphism

Polymorphism is considered as the major obstacle of solid active pharmaceutical ingredients (APIs) when one crystalline chemical can occur in more than one crystal form having different properties like solubility, stability, absorption and bioavailability.[3,10] Through the product handling, the manufacturing conditions (solvent, temperature and pressure) may affect the polymorphic phases and coexist into the drug product which could crystallize out/ precipitate in solution/ formulation and varies the bioavailability.[10,13] When the drug molecules alter to a much more soluble/ bioavailable form then the actual dose of polymorphic drugs could be detrimental/ lethal. Ghielmetti et al. reported that the toxicity or LD50 value of nystatin antibiotic was significantly varied because of their polymorphic forms.[14]

1.2.3. Particle size

Another common problem of drugs molecule is particle size. The pharmaceutical properties of a drug for example solubility, rate of dissolution, uniform distribution, suspendability and permeability can be significantly influenced by increasing or decreasing of the particle size.[10,15]

Thus, it is very essential and required to formulation of drug delivery with organized polymorphic form as well as the precise crystal size and the product solubility.

1.2.4. Route of administration

The active substance needs to dissolve or be solubilized in aqueous media for intravenous formulations to become bioavailable or applicable to the patient due to the irrespective of the intended route of administration. Liquid formulations have more advantageous due to the dosing flexibility and a reduced risk of choking. They can also be useful in other populations, such as geriatric patients with swallowing difficulties, or in a palliative setting. Stability and palatability, parameters are considered as possible disadvantages of liquid formulations.

1.3. Techniques for formulating and delivering sparingly water-soluble drugs

A variety therapeutic approaches to address the sparingly water solubility of a drug can be systematized into three classes according to the nature of the modification involved such as the chemical, physical and administration techniques. These strategies can be used individually or mutually. In the last decades, various approches have been taken for improving the formulation and delivery of low water-soluble immunosuppressants, prostaglandins and antineoplastic agents.[6,16,17] It is very interesting that colloidal systems indicate a more current alternative for the formulation of sparingly water soluble drugs which can be involved into physical or chemical modifications.[11] The carrier-linked prodrugs where the prodrug moiety is covalently linked with the parent drug and another one is bioprecursor prodrugs where the parent drugs are modified with functional groups by enzymatic reactions, hydration or redox reactions. The prodrug based nanoparticles are used in drug loading/adsorption and the use of small drug particles can involve in nanocrystals. Finally, micelles, nanoemulsions, solid lipid nanoparticles or liposomes are included by the surfactant and lipid formulations. A schematic representation for these formulation strategies is illustrated in Figure 1.2 and is proposed to highlight the variety of proven approaches available to those working with low water-soluble drugs.[16]

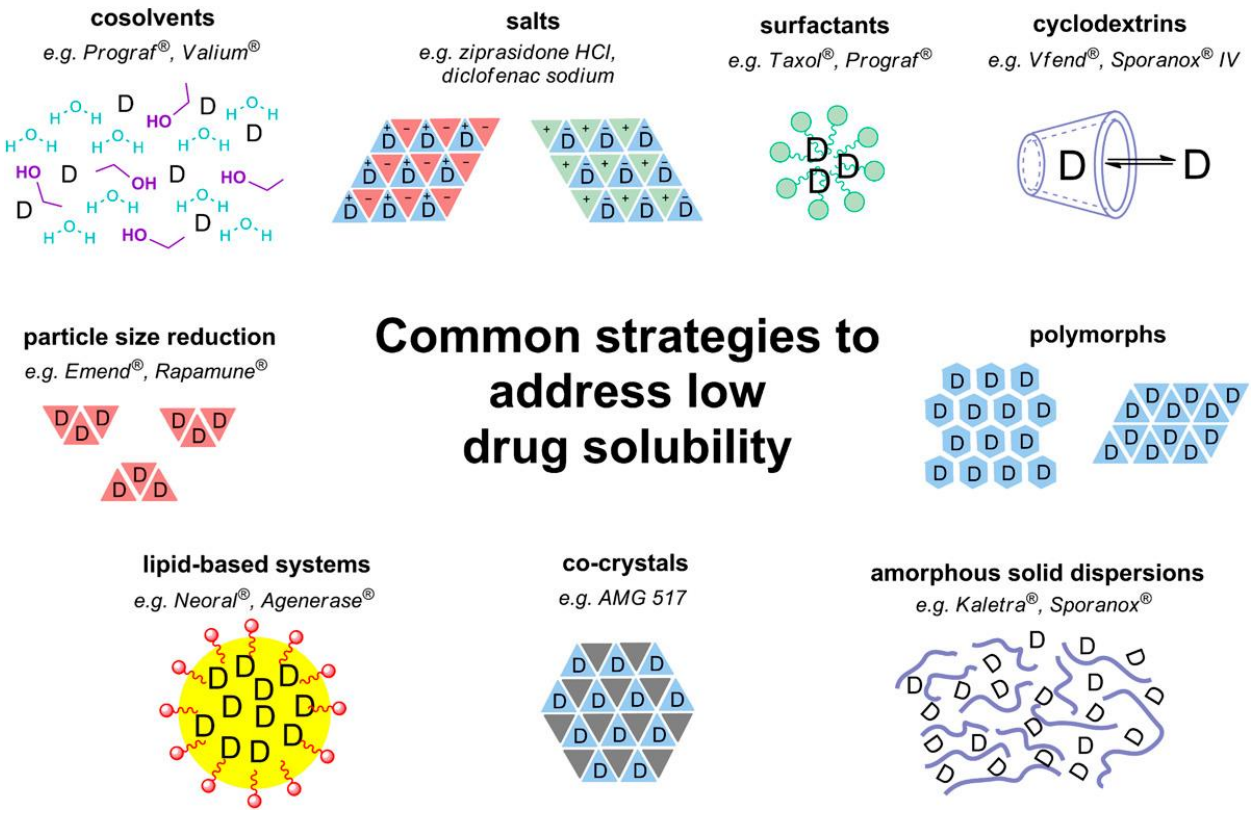

Figure. 1.2. Schematic diagram illustrating the common strategies currently used for poor drug solubility in drug design and development. Figure reproduced with permission from ref.[16]

1.4. Ionic liquids (ILs) as potential components in DDS

In previous reports, ILs were considered as used for catalysts and media of reaction for extractions and separations, as electrolytes in electrochemistry, as applicable in biomass convention, biotransformation, in nanotechnology, lubricants or propellants fluids, and many other fields. In recent years, ILs were considered as an environmentally safe and green solvent, as well as comprised of biodegradable, biocompatible, and natural ions, like amino acids and choline, or ions with recognized biological activity. Therefore, ILs have attracted promising attention to address the potential challenges of solid pharmaceuticals for the effective drug delivery in the medical field. Because of many drug molecules reveal polymorphism, poor solubility, permeability, and bioavailability profiles, which leads to clinical failure and pursue the new approaches to solubilize and/or formulate them. Because of the surprising solvating power, ILs can be improved the solubility of various sparingly soluble drugs and significantly improves the drug penetration over biological obstacles to the improvement therapeutic efficacy.

The literature analysis that has identified the leading directions and driving forces of the current research in the field is shown in Figure 1.3.[18]

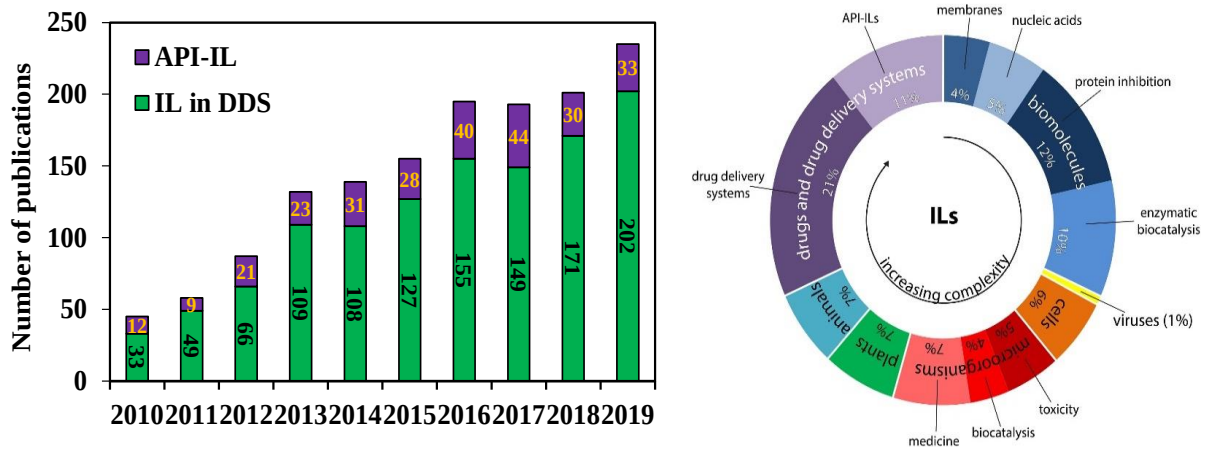

Figure 1.3. ILs as component of drug developments. A) Publication frequency of the term "Ionic Liquids in drug delivery system" and "Active pharmaceutical ingredients ionic liquids" found from Web of science® database. B) Active research directions for studies on biological activity of ionic liquids published in 2017–2018 (for illustrative purpose only). Figure B reproduced with permission from ref.[18]

1.5. What are ILs?

For over a century, ILs have been an interesting topic of scientific researchers and risen the attention in numerous fields of modern science since the mid-1990s.[19] ILs are considered an interesting class of tunable as well as green designer solvents for more than two decades. ILs are defined as stable organic salts that are consist of organic cations and anions through typically liquid lower 100 °C.[19] As a unique family of "green solvents", ILs are notable chemical compounds and explore their applications in different fields of modern science due to their extremely tunable and extraordinary physico-chemical properties such as exceptional thermal and chemical stability, extremely low flammability, low vapor pressure, non-volatility, recyclability, high ionic conductivity, and multiple solvation capabilities through organic and inorganic molecules.[20,21] The synthesis of ILs is not so difficulties, even in large scale. The modification of ILs structural or physico-chemical properties mainly depends on the structure of either cation, anion or to the substituents on the cation or anion.[3,22] Hence, a huge number of ILs are possible to synthesize by altering the cation or anion of ILs (Figure 1.4).

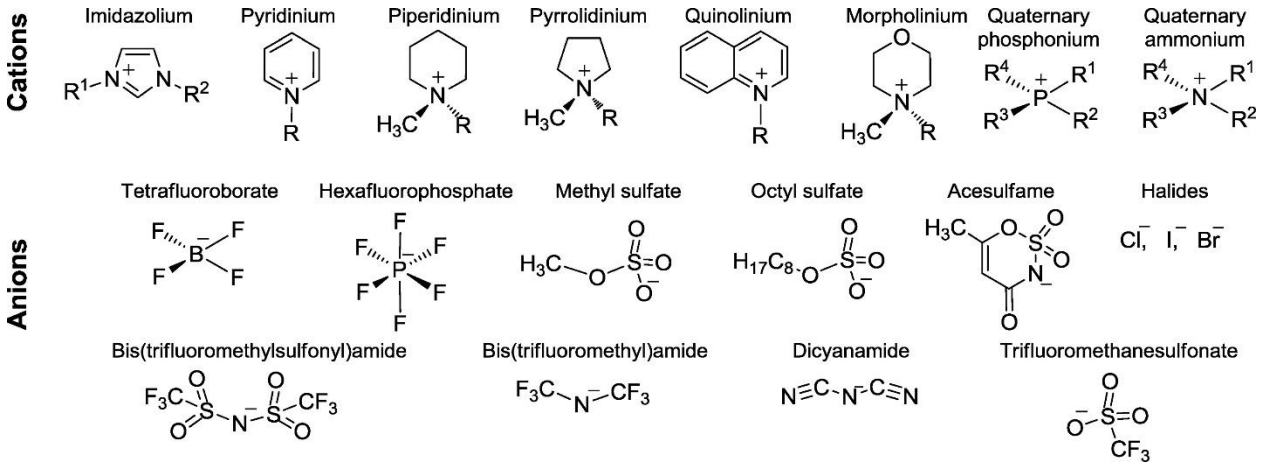

Figure 1.4. Various examples of cations and anions usually used in ILs. Figure reproduced with permission from ref.[10]

However, ILs have been known for all over the past few decades. The first appearance of IL was in 1914 by Paul Walden et al. and reported about the physical properties of ethylammonium nitrate ($[EtNH_3][NO_3]$).[13] Hurley and Weir reported that a room temperature ionic liquid could be synthesized using aluminum chloride ($AlCl_3$) with 1-ethylpyridinium halides, mostly the chloride, at a mole fraction of $X(AlCl_3) = 0.66$ (1:2 ratio).[23] However, the $AlCl_3$-based ionic liquids are hygroscopic in nature, so they have limited use in various applications. Therefore, the air- and water-stable ILs have great attraction to further interest for the use in various fields. The first air and water stable imidazolium-based IL was reported in 1992 by Wilkes et al. which challenged the previous concept of ILs. After this report, the quantity of air- and water stable-based ILs was significantly increased.[24] In 1998, Davis et al. firstly reported "functionalized ionic liquids" as a novel class of IL in wherever the cation derived from the antifungal drug miconazole.[25] Recently, ILs are reflected to be potential candidate as green or designer solvents due to their advantageous toxicity, flexibility and variability, hence redefining the definition of ILs is low melting salts below 100 °C through an boundless set of tunable properties including volatility, biological activity, instability, and flammability.[3,10,22]

1.5.1. Fundamental physico-chemical properties of ILs

Due to the extraordinary physicochemical properties of ILs over conventional organic solvents, the ILs are measured to be a potential candidate for different applications. But the desirable physico-chemical properties of ILs are designed via considering the nature of cations as well as the anions. Some typical structures of cations and anions are revealed in Figure 1.5. Among them, four main categories of cations like imidazolium, ammonium, pyridinium or

pyrrolidinium, and phosphonium that are often used by pairing with various anions in the design of ILs.[10,22] The imidazolium-based ILs can be used as solubility promoters, solvents and catalysts because of their easy synthesis, low stability and viscosity in redox reactions.[26,27] The main drawbacks of a long alkyl chain of imidazolium-based IL are higher toxicity in the biological applications.[18] The pyridinium-based ILs are more active in catalytic reactions but poor regioselectivity in palladium-catalyzed telomerization and Diels-Alder reactions.[20,26] The quaternary ammonium-based ILs have shown lower toxicity than imidazolium-based and pyridinium-based ILs in biological applications. Due to the lower viscosity and melting points, these ILs are used as electrolytes.[10,20,26] Recently, quaternary ammonium-based ILs have been used to improve the antibiotic activity and topical drug delivery.[10] In addition, these ILs are considered as appropriate solvents for pharmaceutical processing which act as substitute to the conventional solvents in pharmaceutical industries. The ILs of Phosphoniums are considered as more thermally stable than imidazolium and ammonium-based ILs, even at high temperature (>100 °C). Recently, these ILs are used in several reactions as solvents and catalysts and more recently for the CO_2 capture.[10,20,22] However, ILs have been highly used in variously application including biocatalysis, biomolecule purification, bio fuel production, energy conversion, anti-microbial agents, pharmaceutical drug delivery, drug formulation and so on.[3,20,28,29] Hence, ILs have remarkable physico-chemical properties such as excellent thermal and chemical stability, low flammability, non-volatility, negligible vapor pressure, recyclability, high ionic conductivity, and multiple solvation capabilities with organic and inorganic compounds.[20,30–33] The influence of the physicochemical characteristics on ILs behavior is briefly discussed below.

Viscosity: Viscosity is known as vital properties of ILs for different applications. It depends on the van der Waals interactions and hydrogen bonding capacity of a material. In the case of ILs, the anion structure was believed to have a significant effect on the viscosity, while a cation showed minor effect. It has been reported that, the presence of a specific structure in the anion could increase the viscosity significantly such as an aromatic ring.[19,21,34] If the ring number in the cation could increase, it showed the increase of viscosity. It may be due to the increase of cation-anion interactions.[24] The viscosity behavior in ILs with altering the cation/anion remain interesting that need further substantiation with a theoretical modelling.

Ionic Conductivity: The ionic conductivity of ILs depends on the viscosity as well as the molecular weight and the size of the ions. If the mobility of ion decreases that will cause to low or limited conductivity. Therefore, less ionic interaction and more delocalized charge will lead

higher conductivity. If the size of cations increases, there may be possibilities of decreasing conductivity of ILs. It has been reported that having a heterocyclic or small molecular weight structure in cations showed a high ionic conductivity in ILs.[34–36] However, no obvious relation can be found for the anions used in the system.

Density: Both cation and anion have influences on the density of ILs. In general, the density of an IL inversely proportional to the bulkiness of the cation grows. The reason may be due to the poor crystal packing of the ILs formed between the bulky cation and the weakly complexing symmetrical anion. In another case, the change of anion such as replacing the hydrogen atom with heavier elements (F, Cl, Br) increases the density of ILs. It has been reported that the temperature has a significant effect on the density of ILs, as the temperature changes from 293 to 313 K, the density decreases linearly as the temperature increases.[20,36,37]

Thermal Stability: One of the important parameters of ILs is the possession of excellent thermal stability after conversion to ILs from their origin (cations or anions). Many biologically active compounds (active pharmaceutical ingredients, APIs) have been reported thermal unstable, but become stable upon formation of ILs.[38] Numerous RTILs with high thermal stability have been also reported, like, Wilkes et al. described that $EMIBF_4$, $BMIBF_4$ and 1,2-dimethyl-3-propylimidazolium bis(trifluoromethylsulfonyl)amide (DMPITFSA) are stable up to temperatures of 445, 423 and 457°C, respectively.[39] Nevertheless, Endres et al. also reported that as high temperatures are only allowed by most liquids for a little time and that long time contact to such high temperatures unavoidably leads to decomposition.[40]

Melting Point: The melting point is an essential physical property for a molecule that has been used as unique identification. The melting point of ILs is considered to be significant because of the ILs have a wide liquids range measured by their low melting point as well as high decomposition points.[30] Most importantly, the ILs solubility in aqueous and organic solvents is considered to be strongly interrelated with their melting point.[20] It has been reported that, the freezing point arises at the same temperature as the melting point.[20,30,31] However, the thermal decomposition is intensely dependent on the structure of ILs. In general, the imidazolium cations incline to be thermally more stable than that of the tetra-alkyl ammonium cations. High thermal stability can be delivered by certain kinds of anions like $TFSI^-$($[(CF_3SO_2)_2N]$) and $BETI^-$($[C_2F_5SO_2]_2N$). The relative stability of anions are the following order PF_6^->$BETI^-$ >$TFSI^-$>$CF_3SO_3^-$>BF_4^->Me $[(CF_3SO_2)_3C]^-$>I, Br, Cl[3,28–30,34]

1.5.2. Surface active ionic liquids (SAILs)

Surface-active compounds or surfactants are an interesting class of chemical compounds, which are used in different areas of modern industry, like pharmaceutical, food, petroleum, and cosmetics industries. The main properties of these compounds are capable to decrease the surface and interfacial tensions and also form and stabilize the oil-in-water or the water-in-oil or non-aqueous emulsions.[41,42] Usually, these are known to be amphiphilic molecules, which contain both hydrophilic and hydrophobic moieties, a long alkyl carbon chain containing part is called apolar, where the polar part is more variable, which can be ionic (anionic or cationic) or non-ionic.[41–43] The exceptional market need for surfactants is presently met by numerous synthetic, principally petroleum based or chemical surfactants. They are generally known to be toxic as well as non-biodegradable to the environment. These compounds also formed unstable micelles in aqueous media, when using a large amount of surfactant.[44] To address these issues, researchers are trying to improve their surface-active properties by varying the physi-co-chemical conditions. Recently, surface active ionic liquids (SAILs) as environmentally friendly surfactants have attracted considerable attention as the promising substitutes of conventional surfactants because of their exceptional and favorable properties, for example high thermal stability, negligible vapor pressure, biodegradability, non-volatility, and non-flammability[42,45,46]. It is well known that various ILs are dependent on the mixture of cations and anions which show surfactant like behavior and also place them in the category of ionic surfactants and are usually termed as SAILs.[47–49] These SAILs occasionally have better surface active properties as likened to conventional surfactants and also have been used for creating various kinds of molecular assemblies like reverse micelles, normal micelles, and vesicles.[43,50–52] At first, Sirieix-Plenet and Bowers et al. investigated out the amphiphilic nature of ILs in water in 2004.[53,54] Sirieix-Plenet et al. also described the formation of micelle of IL C_{10}MIm-Br in water.[53] The authors detected that the IL aggregates and forms micelles at low concentrations, but at higher concentrations it assumes to be a multifaceted structure with interpenetrated domains of the water and electrolyte. In this point of view, SAILs have been used as possible alternative to substitute conventional ionic surfactants for different industries.

1.5.2.1. Characterization of SAILs

In recent years, the aggregation behavior of SAILs in water is investigated by different experimental and computational technics such as conductivity measurements, surface tension (ST), potentiometry, fluorescence probes, ultraviolet-visible spectroscopy (UV-Vis), nuclear

magnetic resonance spectroscopy (NMR), isothermal titration calorimetry, mass spectrometry, light scattering, molecular dynamics simulations (MD) and small-angle X-ray and neutron scattering (SAXS and SANS) among others.

Surface activity and thermodynamic properties: According to the colloidal and surface chemistry, the concentration of surfactants above which micelles form and also all added surfactants to the system are certain in micelles is defined as the critical micelle concentration (CMC). The characterization of CMC and wide thermodynamic studies permit to identify the exact features of SAILs which affect the properties of these ionic liquids. Numerous reports indicated that the CMC extremely dependent on the structure of the ionic liquid, and a linear connection between the number of carbon atoms on the alkyl chain and the logarithm of the CMC has been performed.[41,42,51,52,55] 1-Alkyl-3-methylimidazolium salts formed spherical or elongated micelles in water at low concentration but the shape of the micelle has been changed with higher concentrations.[43,56,57] For example, different self-aggregation of [C_{12}mim]Br using a negative stain transmission electron microscopy and a cryogenic-transmission electron microscopy (Cryo-TEM): **(a)** spherical micelles (about 350 nm in diameter) and **(b)** rod like micelles (about 200 nm in width) were found at the ionic liquid concentrations of 60 and 560 mmol/L (mM) respectively; at 930 mM **(c)** unilamellar vesicles (about 960 nm in diameter) were observed in Figure 1.5 .[58] This is the first observation for the formation of stable vesicles of IL surfactants by single tails in aqueous solutions without any additives. In this thesis, the surface tension and conductivity were used to investigate the aggregation behavior of SAILs. In all technics, the CMC was calculated at the break point (the intersection of the two linear fittings of data points), but other parameters can be gained also from these measurements.

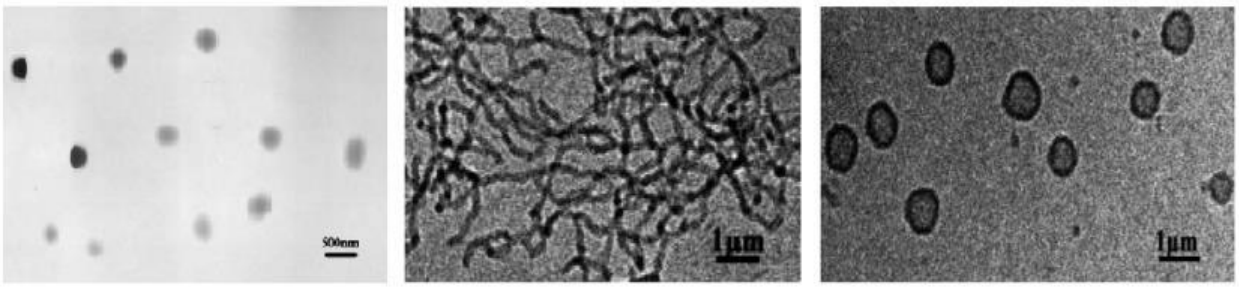

Figure 1.5. Aggregates of [C_{12}mim] Br in aqueous solution at different concentrations. Figure reproduced with permission from ref **.**[58]

Conductivity measurements. The aggregation behavior of ILs is evaluated with investigating the electrical conductivities of aqueous solutions of IL. Depending on the self-aggregation of

the IL surfactant, individual ions and the aggregates have different mobilities. A plot of the ionic liquid concentration versus the specific conductivity (k) two unlike linear regimes could be usually identified that permitted to calculating the CMC values by their breaking point as per Williams methods. There are two reasons for this curve shape: the lower mobility of micelles as related to that of free ions and decreasing the current charge on micelles, because of counter ion binding on the micellar surface, which indicates a smaller slope in the post-micellar area of the conductivity isotherms.[41,55] The degree of counterion binding (β) can be assessed from the ratio of the slopes above (S_2) and below (S_1) the CMC (equation 1.1) which specifies the number of anions on the micellar surface.[41] A lower value of the latter resembles to a higher CMC because of the lower number of anions bound to the surface of the micelles.

$$\beta = 1 - {}^{S_2}/_{S_1} \quad \text{..... (1.1)}$$

In addition, the Gibbs energy of micellization (ΔG_m^0) can be determined from the conductivity measurement (equation 1.2).

$$\Delta G_m^0 = RT(1+\beta) ln X_{CMC} \quad \text{..... (1.2)}$$

Where the argument of the logarithms is the mole fraction of the surfactant and the water concentration (W) is taken as 55.4 mol/L (M).

Surface tension measurements. The surface tension curve signifies a measure of the cohesive forces at the gas-liquid boundary. This result is very essential to identify the CMC and inspecting the adsorption behavior at the gas-liquid interface. Because of adsorption of ionic liquids at the surface, the surface tension initially increases with decreasing ionic liquid concentration, till a constant value is found when the surface is saturated.[41,59] Generally, the obtained CMC values are thoroughly slightly less than those of the conductivity measurements. The efficacy of the surface tension reduction (Π_{CMC}) provides how much the SAILs decrease the water surface tension which is determined according to the following equation (equation 1.3).

$$\Pi_{CMC} = \gamma_o - \gamma_{CMC} \quad \text{..... (1.3)}$$

Where, γ_o and γ_{CMC} are represent the surface tensions for pure solvent and solutions at CMC, respectively.

The degree of packing of surfactant molecules at the interface can also be related to the effectiveness. The Gibbs adsorption isotherm (equation 1.4) can be used to determine the

average area per molecule exist in at the surface (Amin), if the structure design corresponds to a monolayer.[41,60]

$$A_{min} = \frac{10^{18}}{\Gamma_{max} \cdot N_A} \quad \text{..... (1.4)}$$

Where N signifies Avogadro's number and the surface excess concentration (Γ) is calculated with the following equation (equation 1.5).[42,61]

$$\Gamma_{max} = -\frac{1}{nRT}\frac{\partial \gamma}{\partial lnC} \quad \text{..... (1.5)}$$

Where is the slope of the curve surface tension vs lnC before the CMC (concentration is expressed in mol/L, and R is 8.31 J/mol·K).

A lower A_{min} value indicates to a nearer packing of monomers at the interface.[42,55,61] The value could be used for determining the shape of the formed micelles or aggregates allowing to the packing parameter (P) which contains the volume (v) of the hydrocarbon chain fixed in the hydrocarbon core of the aggregate and the maximum effective length (lc) that the chain can adopt, designed affording to the Tanford equation (equation 1.6, 1.7 and 1.8).

$$v = (27.4 + 26.9 * n)10^{-3} nm^3 \quad \text{..... (1.6)}$$

$$l_c = (0.154 + 0.1265 * n)10^{-3} nm \quad \text{..... (1.7)}$$

$$P = \frac{v}{a_0 l_c} \quad \text{..... (1.8)}$$

In the equation 1.6 and 1.7, n represents the actual number of atoms in the hydrophobic core of the micelle. In the equation 1.8 the value indicates the cross-sectional area engaged by the hydrophilic group at the solution-micelle interface and it has been approximated with A_{min}.

Thermal properties: The properties of materials which altered with temperature are then investigated by Thermal Analysis (TA). Thermal properties such as decomposition and melting points of ionic liquids are one of their great properties which define the liquid range of the compounds, and consequently, their range of applications. In which temperature the SAIL becomes liquid known as the melting point, while the decomposition point determines the upper operating temperature for that fluid. An inorganic anion containing samples is more likely to be solid at room temperature than those with an organic anion and generally many of the SAILs are highly thermally stable, often decomposing around 300-400 °C with minimal vapor pressure below their decomposition point.[55,62] Hypothetically, the freezing point arises at the same temperature as well as the melting point arises but in general ILs show actual super

cooling while being cooled from comparatively high temperature. The procedure of lowering the temperature of a gas or a liquid lower its freezing point without it flattering a solid is known as supercooling as well as undercooling. Several studies showed that thermogravimetric and differential scanning calorimetry analysis (TGA-DSC) are used simultaneously for studying the thermal properties of pure surface-active ionic liquids.[39,41,55,62,63] TGA can offer information about physical phenomena, like second-order phase transitions, such as vaporization, absorption, sublimation, adsorption and desorption and also about chemical phenomena like chemisorption, decomposition, desolvation (especially dehydration), and solid-gas reactions. Hence, TGA is a useful tool to measure the decomposition point of the synthesized surface-active ionic liquids.

An important thermal analysis known as DSC, which provides information on the phase transitions, i.e. transition temperatures (like glass transition temperature, freezing point, and melting point) or enthalpies. The DSC curve displays a typical shift in the endothermic direction at the glass transition of amorphous substances with the increasing of specific heat at 0.1 to 0.5 Jg-1·K-1. Glass transition occurs in the case of a hampered crystallization of the ionic liquids and verification takes place when a liquid is cooled sufficiently fast to elude crystallization. At the freezing point, the process is exothermic whereas the melting process is endothermic.[62,64] The melting curve is used to estimate the heat of fusion and the melting point. In the case of pure substances, the melting peak at low temperature is nearly a straight line, the melting point resembles to the onset. But in adulterated samples the concave shape melting curves are categorized by the temperatures of their peak maxima. When the sample weight does not decrease considerably over the course of the peak, an endothermic peak in a DSC heating curve denotes the melting peak. Organic substances create a melt that on cooling solidifies to a glass or crystallizes with an exothermic peak. DSC also can provide some information about liquid crystals[64]. Liquid crystals are differentiated as monotropic and enantiotropic which depends on the thermodynamic stability. The enantiotropic states are stable and can be formed in a certain temperature range during both the heating and the cooling scans. On the other hand, the monotropic mesophases are only metastable and are observed because of supercooling, so they can only be detected during the cooling process.

1.5.3. Applications of ILs in different fields

Due to the tailored made properties of ILs, they have been sustained to be used in numerus fields over the last few decades. A list of fields of ILs application has been given below, but not all such as-

- ILs as liquid lubricant in a different range of systems have established that these materials can display outstanding protection against wear and considerably decrease friction in the neat state. ILs are also used as non-polar base oils, substituting conventional anti-wear additives, with exceptional performance of the neat IL being preserved.
- ILs in analytical chemistry such as chromatographic/capillary electrophoretic (CE) separation, sample preparation, and detection.
- ILs as electrolytes in lithium batteries, aluminium electroplating, and in electrochemical capacitors.
- ILs as green solvent for lignin or lignocellulosic biomass deconstruction.
- ILs or IL-based polymer electrolytes in batteries.
- ILs in separation and extraction of bioactive compounds from plants.
- ILs for new reaction media may suggest a suitable solution to both the solvent emission and the catalyst reusing problem.
- ILs as solvents in polymerization procedures.
- ILs in electrochemical sensing systems
- ILs for energy, materials and medicine.
- ILs as extracting agents for critical metals.
- ILs for synthesis of inorganic nanomaterials.
- ILs as plasticizers for poly (methyl methacrylate).
- ILs as drug delivery system (DDS)

1.5.4. Applications of ILs in DDS

ILs are considered as extraordinary chemical compounds which novelty multiple applications in various fields of modern sciences. Because of their extra ordinary physicochemical properties and highly tunable nature, ILs were developed to be important components of in the areas of synthesis, catalysis, electrochemistry, extraction, analytics and biotechnology.[65] It is newly emerging the biological activities of ILs and their application in pharmaceutics and medicine. It has been well described that most pharmaceutical industries are facing

unprecedented challenges for designing the smart drug delivery systems of poorly water-soluble drugs over the years due to their limited solubility, stability, bioavailability, and polymeric transformation.[20] These problems are intensified if drug substances are poorly soluble in water as well as pharmaceutically approved solvents. It is very important concern that poorly soluble drugs cause to low drug absorption and bioavailability through all the methods of drug delivery.[66] The oral delivery of many drug substances is hindered due to their water insolubility as well as lipophilicity such as tamoxifen, paclitaxel, docetaxel etc.[19] Furthermore, nucleoside based drugs are sparingly soluble in traditional solvents that are used for the formulation of essential drugs. It was described as a major concerning issue in the pharmaceutical industries.[4,26,32,35,67] To solve this issue, ILs are used as solvents, co-solvents, surfactants and/or formation of API-IL. The possible application of ILs was initially hindered due to their toxicity and antimicrobial activity in pharmaceuticals. Later, it was discovered the use of ILs as greener as well as designer candidates in biological applications like enzymatic processes, whole-cell biocatalysis and protein stabilization.[68–72] Recently, ILs have been considered to be a potential candidate in pharmaceutical fields such as the formation of microemulsion, API-ILs, ILs/IL mixture as solvents or co-solvents for the development and refinement of pharmaceutics and for low soluble drugs, and DDS.

1.5.4.1. ILs are considered as alternative of conventional organic solvents

It is well established that a lot of therapeutically active drugs which are in either solid or in crystalline forms that are sparingly soluble in conventional organic solvents.[4] Because of these problems, the formulation scientists have faced very challenging for their formations. Additionally, the low solubility of these molecules generally associated with the poor absorption as well as poor bioavailability upon oral administration. It is well established that, many hopeful drugs which are poorly soluble in conventional solvents not ever enter a formulation because of their low solubility and difficulties in the delivery system in pharmaceutical industries and in modern medicine.[3,34] There are various strategies available and described in literature to improve the solubility of sparingly water soluble drugs. One of the best way to overcome the problems and enhance their solubility is to use of excipients as polar organic solvents, like N,N-dimethylformamide (DMF), pyridine, and dimethyl sulfoxide (DMSO).[34,73] However, these solvents are not considered as an inert vehicle as well as they have many disadvantages like they are flammable, volatile, and toxic. It has been very essential to remove from final products when these types of solvents are used in reaction media for synthesis of drugs.[74,75] But the recovery and reuse steps are not convenient. Because of highly

tunable and exceptional physico-chemical properties of ILs, it is extremely required for ILs to be the potential alternative of conventional organic solvents.[3,28,29] Moreover, ILs are also capable to dissolve complex molecules like biologically active molecules including protein, nucleotides and amino acids under milder conditions due to their tailor-made properties. The extra ordinary properties of ILs permit the possibilities of tailoring therapeutic solvents or the use in the synthesis for new drugs with precisely desired properties that are efficiently restricted when water or molecular organic solvents are used. However, ILs are widely used as solvents in chemical processes such as synthesis, separation and catalysis have presently become important. Till to date, IL remains one of the best dynamics as well as remarkable fields of researches with over 7000 contributions in 2018 alone. A literature search for IL in Scopus ® database has shown that only 3 works had in 1990 and 609 works in 2000, which rapidly increased in 2018 for 7000 works (Figure 1.6).

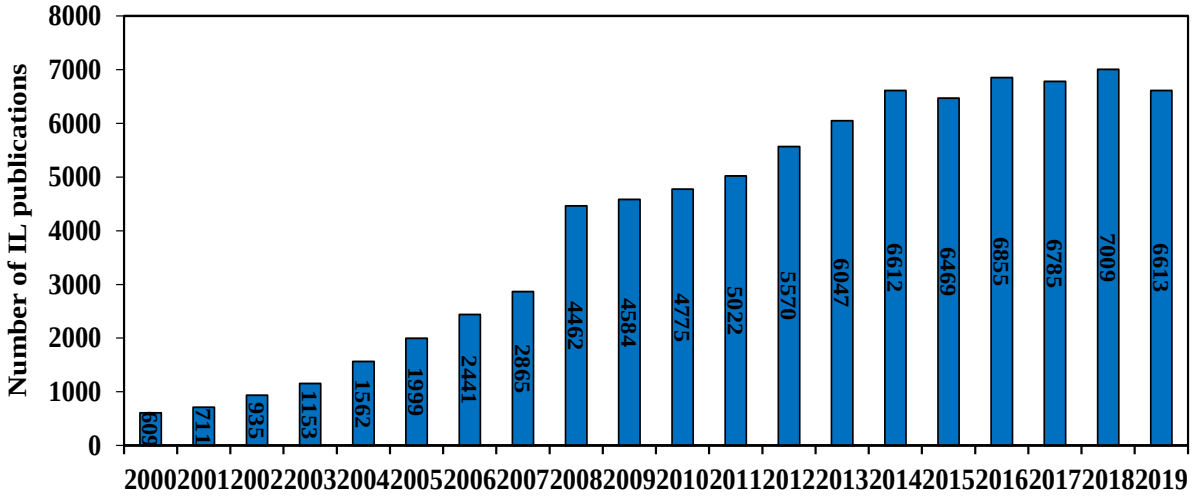

Figure 1.6. Publication frequency of the term "Ionic Liquids" found from Scopus® database

1.5.4.2. ILs are considered as alternative of conventional surfactants

Recently, SAILs have fascinated great consideration as probable alternatives to conventional surfactants due to their exclusive tailor-made properties. It has well reported that most of the ILs depend on the combination of anions and cations displays surfactant like nature, which places them in the class of ionic surfactants and are usually termed as SAILs.[41–43,59,76] These type of ILs sometimes show better surface active behavior than that of traditional surfactants and are used to form supramolecular nano- to giant aggregates such as vesicles, multilayered vesicles, micelles, and wormlike micelles, and also used in chemical, industrial, or pharmaceutical applications.[56,59,64,77,78] SAIL compounds are also finding an extensive area of applications in DNA stability, in micellar catalysis, in encapsulation of drugs, and in

enzyme/protein stabilization in detergent developments. Amphiphilicity of ILs can be improved by linking medium to a longer alkyl chain to cation and anion or both and used as surfactants. In the last few years, several research groups have been established and examined the self-assembling nature in aqueous or also IL media of various types of SAILs which are similar to the conventional surfactants such as cationic, anionic, zwitterionic and biamphiphilic or catanionic.[47,48,78–81] The SAILs have also numerous advantages than the conventional surfactants of analogous alkyl chain length. The SAILs of imidazolium have been used to stabilize the metal nanoparticles through surface charge covering.[81,82] The head group of imidazolium has decreased as well as stabilizing capability in chemical and photochemical synthesis of gold nanoparticles through oxidizing itself into ketone.[47,81,83] The bio-based amino acid derived SAILs have displayed more advantages such as in the synthesis of shape-controlled nano-compounds and in the mitigation of unsafe algal blooms.[84] These types of SAILs have been used to improve the emulsifying capacity particularly in the preparation of IL based microemulsions, wherever the traditional anionic surfactants are insoluble.[84,85] Thus SAILs are considered to be possible alternate to substitute conventional surfactants for preparation of microemulsions.

1.5.4.3. Biocompatibility and Biodegradability of ILs

Biocompatible ionic liquids (Bio-ILs) are considered as an eco-friendly group of ILs advantageous in applications extending from the electrochemical to the biomedical fields. Before going to use of ILs in pharmaceutical application, it is important to confirm the biocompatibility of the molecules. Because of the questioning green nature of usually used ILs, the synthesis of Bio-ILs has been increasing. The best technique to incorporate Bio-ILs utilizes both anionic and cationic counterparts obtained from biocompatible materials, such us amino acids (AAs), glucose, non-nutritive sweeteners, and carboxylic acids.[65,86–89] The novel ILs are synthesized by utilizing these bio-renewable and natural compounds which are considered as promising components as to both environmental and financial concerns strongly connected with ordinary methods. Fukumoto et al. were the first pioneers that depicted the fruitful synthesis of room temperature ILs (RTILs) by joining the imidazolium cation with 20 different natural AA.[90] In another study, imidazolium based ILs showed low/negligible toxicity towards Caco-2 cells.[91] It has been reported and widely accepted that the biocompatibility/toxicity of any ionic liquids is largely dependent on the cation; of course the anion also has a role.[92] For example, ionic liquids comprising ammonium and alicyclic cations (piperidinium, morpholinium, and pyrrolidinium) display lower toxicity than those with imidazolium and

pyridinium.[30] Introduction of polar hydroxyl group/ nitrile/ether functional groups into the alkyl chain can essentially reduce the toxicity.[28,29,34] In a decades ago, a noticeable number of studies have demonstrated that utilizing choline as the cation offers Bio-ILs with biodegradability and less toxicity.[65,86–89] Choline can be attained from natural sources and is generally combined with B-complex vitamins. In addition, cholinium cations have numerous functions, such as memory and muscle control, because of its function as a precursor to the neurotransmitter acetylcholine. Cholinium cation additionally incorporates the head groups of two classes of cell membrane- plentiful phospholipids, to be specific, phosphatidylcholine and sphingomyelin. The most desired anion should be amino acids that are the most rich groups of organic compounds in nature well known as biodegradable, nontoxic, and biocompatible.[26,86] It has been reported that many valuable and essential chemicals are poisonous, many pharmaceutically accepted excipients for example, DMSO and nonionic surfactants (e.g., Tween-80, T-80) show comparable toxicities as noticed from ILs.[34] That is why consideration ought to be paid in the choice of ILs for DDS, on the grounds that the toxicity of ILs relies significantly upon their cations and the alkyl side chain.

1.5.5. ILs techniques in DDS

The main problems such as polymorphism, solubility and bioavailability of a drug molecule are overcome by the successful utilization of ILs in DDS. All ions of ILs can freely and/or concurrently present a particular functionality or property adding tunability or adaptability to the plan of new functional materials. Proper changing of ion can modify the specific physiological and biological properties of ILs.

1.5.5.1. ILs are considered as excellent solubilizing agents

Majority of the failures in the new drug improvement has been assigned to incorporate poorly soluble drugs into delivery systems. Also, low solubility can prompt to poor bioavailability in the problematic drug delivery. In this regard, it is highly essential to find replacements way like finding excipients which improve drug solubility. To address this issue, ILs are considered as solubilizing agents in drug delivery systems. In the mid-1990, ILs have been usually known as designer solvents because of remarkable solvent abilities.[19] The excellent solvating power of ILs can be enhanced the physico-chemical properties of solid form by converting into the liquid form. Many excipients are extremely challenging to dissolve with physiological fluids and/or FDA approved solvents. Recently, the use of organic solubilizers is an indisputable challenge for an effective drug delivery because of their toxicity and contamination ability of

pharmaceutical products.[10,28,93] ILs can be designed to dissolve hard-to-dissolve drugs with increased shelf stability and drug concentration for delivery which as considering a suitable alternative of organic solvents, (Table 1.1).[22] The mechanism of dissolving ability of ILs has already reported by investigational result and/or molecular dynamic simulations which recommend that the continuous polar system is created in ILs which disrupted into smaller domains so that the ILs solutions are look like with water matrix where the ionic filaments are merged.[94] The structure of IL filaments in the water solution can be stabilized because of the formation of H-bonds among the anions of ionic filaments and water molecules. Claudio et al. reported the snapshots of the equilibrated simulation for revealing the presence of co-aggregates among the hydrophobic solutes and the IL ions in an water solution (Figure 1.7).[94] It has been showed that the vanillin molecules properly mix with water and create cation−vanillin clusters by dispersion forces and other particular interactions, like H-bonds and π−π interactions. Several studies favor the relationship between tremendous solubilizing properties of ILs as well as their capability to create various interactions with the solute.[94]

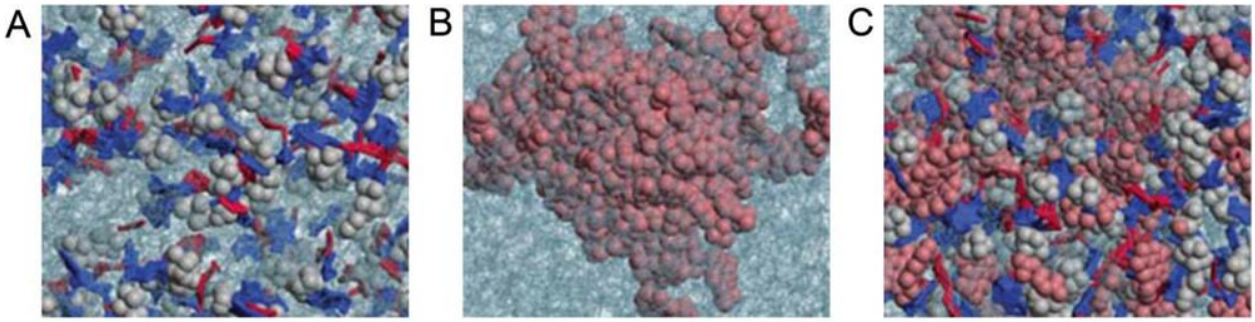

Figure 1.7. Simulation snapshots. (A) IL ([C_4mim][$N(CN)_2$]) in water; (B) Vanilla in water; (C) IL and vanilla in water. (Light green): IL polar aggregates (strands); (blue): anion-water network; (light red) vanillin clusters. Figure replicated with permission from ref.[94]

Normally, ILs may be used as solvents or antisolvents, cosolvents, copolymers and emulsifiers for the development of problematic drugs. The hydrophilic ILs can easily solubilize the hydrophilic drugs, while hydrophobic drug molecules favor hydrophobic ILs.[10] The drugs molecules can dissolve in the IL solution by the influence of both anions and cations even though the effect of the anion is still complex.[95] Also, the solubility is correlated to the alkyl chain length of cations. The solubility promted a decreased with increasing alkyl chain length of cations. Jaitely et al. reported the solubilities of penicillin, dexamethasone, progesterone and dehydro-epiandrosterone in ILs of imidazolium with varying alkyl lengths (C_4, C_6 and C_8).[96] As increased the alkyl length of cations, the solubilities of these drugs are decreased. Hence,

the capability of various ILs to enhance the solubility and permeating ability of sparingly water-soluble drugs that can be a potential strategy of improved absorption and bioavailability of the drugs at the directed site of action.

Table 1.1. Solubility enhancers of ILs in drug delivery. Table reproduced with permission from ref.[10]

Drug	Activity	IL
N-acetyl-L-cysteine	antioxidant	$[C_2mim][OTf]$, $[(C_6)_3C_{14}P][Cl]$, $[(C_6)_3C_{14}P][NTf_2]$
acetaminophen	analgesic	$[C_4mim][BF_4]$, $[C_8mim][BF_4]$, $[C_4mim][PF_6]$, $[C_4mim][Br]$, $[C_6mim][Br]$
acyclovir	antiviral drug	$[C_1mim][DMP]$
albendazole	antiparasitic	$[C_4mim][PF_6]$, $[C_6mim][PF_6]$, $[C_8mim][PF_6]$
amphotericin B	antifungal agent	$[C_2mim][OAc]$, $[C_nNH_3][OAc]$ (n = 4, 6, 8), [m-PEG350-NH_3][OAc]
coumarin	anticoagulant	$[C_{10}mim][NTf_2]$, $[(C_6)_3C_{14}P][Cl]$, $[(C_6)_3C_{14}P][NTf_2]$
4-hydroxycoumarin	anticoagulant	$[C_2mim][OTf]$
curcumin	antioxidant	$[C_4mim][BF_4]$
danazol	steroid drug	$[C_6C_6OCOPy][NTf_2]$,$[C_6C_6OCOPy][N(CN)_2]$, $[C_4mim][PF_6]$, $[C_8mim][PF_6]$,$[C_4miM][BF_4]$
dantrolene sodium	muscle relaxant	$[C_1mim][DMP]$
dehydroepiandrosterone	steroid hormone	$[C_4mim][PF_6]$, $[C_6mim][PF_6]$, $[C_8mim][PF_6]$
dexamethasone	steroid drug	$[C_4mim][PF_6]$, $[C_6mim][PF_6]$, $[C_8mim][PF_6]$
diclofenac	NSAID	$[C_6mim][Br]$, $[C_{12}mim][Br]$, $[C_{14}mim][Br]$
ibuprofen	NSAID	$[(C_6)_3C_{14}P][Cl]$, $[(C_6)_3C_{14}P][NTf_2]$, $[C_2mim][NTf_2]$
etodolac	NSAID	$[C_4mim][PF_6]$
isoniazid	antituberculosis	$[C_{10}mim][OTf]$, $[(C_{10})_2(C_1)_2N][NO_3]$
itraconazole	antifungal drug	$[C_6C_6OCOPy][NTf_2]$,$[C_6C_6OCOPy][N(CN)_2]$, [m-PEG350-NH_3][OAc], [m-PEG350 NH_3][C_nCOO] (n = 3, 5, 7, 9)
methotrexate	anticancer	$[C_1mim][DMP]$

1.5.5.2. API-ILs as pharmaceutical in drug delivery:

Approximately 40-70% of FDA-approved drugs are failed to attain the therapeutic delivery due to their polymorphism, limited solubility and poor bioavailability.[3] The active pharmaceutical ingredient–IL (API-IL) is a novel approach to solve the innate difficulties of

several drug candidates. Rogers and co-workers firstly prepared API-ILs in 2007 and obtained [lidocainium][docusate] (LD) API-IL by coupling lidocaine, a extensively used local anesthesia, with sodium docusate, a laxative.[3] A significant enhancement with improved solubility and thermal stability was achieved as compared with lidocaine hydrochloride in the topical analgesia treatment.[3,21,29] It was also reported as the polymorphic conversion, as the ranitidine docusate was a dark red liquid through glass transition temperature T_{glass} of -12 °C. The API-IL of didecyldimethylammonium ibuprofenate from sodium ibuprofen and didecyldimethylammonium bromide was prepared by using the same process, where sodium ibuprofen showed anti-inflammatory on the other hand didecyldimethylammonium bromide has antibacterial as well as anti-inflammatory properties. The prepared API-IL revealed both of cationic and anionic properties and the active ion could be cation and anion or both.[3,29,30,34] Numerus prominent works have been carried out to emphasize the influence of counterion on API-ILs for tuning their various pharmaceutical cocktail properties i.e., solubility, thermal stability, toxicity and bioavailability.[3,17,21,29,97,98] Recently, Rogers and co-workers demonstrated a series of various IL-forming ions such as cholinium, tetrabutylammonium, tetrabutylphosphonium, tetramethylhexadecyl- ammonium cations, and docusate and chloride anions were combined with acyclovir as the counterion to create ILs.[3] All the cholinium-based acyclovir API-ILs showed higher solubility in water as well as simulated body fluids because of the presence of hydrophilic cholinium cation with hydroxyl groups. In addition, Marrucho groups reported the physicochemical and pharmaceutical properties of API-ILs, which are obtained by pairing the biocompatible and the low toxic cholinium cation with various API anions such as niflumic acid, nalidixic acid, 4-aminosalicylic acid, picolinic acid, pyrazinoic acid, and exhibited enhanced solubility and bioavailability as compared with neutral APIs[60].[87]

1.5.5.3. ILs are consider as Skin Permeation Enhancers

Skin is considered as the most essential barrier to shelter the human body as well as offers a suitable route for the pharmaceutical delivery because of numerous advantages, like sustained and controlled delivery, good patient compliance, greater local concentration and avoiding of the first pass metabolism.[98,99] Nevertheless, the quantity of drugs infiltrate through the skin is extremely low because of multilayers of dermal obstacles, particularly the stratum corneum (SC).[98,100] Recently, ILs or ILs microemulsions were found to be able to improve penetration of drugs over the skin ; hence, many research approaches were led to represent their fundamental mechanisms of action.[100–103]Numerus proposed mechanisms were mainly

dependent on the chemical make-up of the ILs. Monti et al. have been reported that the degree of penetration depends on the structure of ILs.[98] The physicochemical properties of ILs are the key factor related to permeation enhancement.[104] Conversely, the mechanism of permeation is tremendously wide-ranging and doesn't represent all IL permeation improvement profiles. All permeation enhancer ILs are classified into hydrophilic and hydrophobic. Hydrophobic ILs can promote separating into the epithelial membrane by means of providing channels, therefore advancing transcellular transport in the lipid sections whereas hydrophilic ILs act via opening tight connections inside the SC, in this way improving paracellular transport acting by increasing fluidization principally within protein and lipid sections.[22] Among the best recorded is the action of 1-octyl-3-methylimidazolium-based ILs which act by upsetting structural integrity by injecting into the membrane .[105] It has also shown that ILs empower the fluidizing ability to cell membranes, especially seen in hydrophilic imidazolium-based ILs and lipid removal in the SC. Recently, the choline and geranic acid based ILs show high penetrability, antibacterial capability and assisted transdermal delivery of proteins and RNAi like cholinium-malic acid based IL acts as a skin penetration enhancer.[95,99,106,107] In the therapeutic or biomedical applications of any technology, toxicity is considered to be a crucial factor. Bio-based ILs can overcome this problem due to their required biodegradation and reduced toxicity profiles. The ILs of choline are recently the most severely investigated .[86,87,89]

1.5.5.4. ILs are in topical and transdermal drug delivery

The solubilizing capacity of ILs is extraordinary and important to their use not only limited to solubilizing agents, but also oral, topical, and transdermal systems for an effective delivery of poorly bioavailable drug molecules. Topical or transdermal drug delivery (TDD) is considered to be potentially safe and non-invasive system than the conventional delivery strategies (i.e., oral, injection and nasal) due to easily usage, painless, avoidance of first-pass hepatic elimination and lessening of side effects.[3,95,98] TDD can be improved the drug bioavailability and reduced undesirable metabolism in the GI tract, oral cavity, and liver.[98] In a pharmaceutical industry, several transdermal drugs are existing in the market such as estradiol, testosterone, fentanyl, nitroglycerin, clonidine, nicotine, and scopolamine.[108] However, in the case of topical and transdermal administration of drugs, pharmaceutical companies have pretended challenges due to the sparingly soluble or insoluble in water and maximum of organic solvents. However, the effectiveness or efficacy of the TDD systems mainly depends on the drugs physicochemical characteristics and the outermost layer of the skin which represents about 10% of total body mass with covering about 1.7 m^2.[26,108,109]

The stratum corneum (SC)-the formidable barrier to the delivery of drug molecules- is structurally well-organized corneocytes with lipid layers.[110,111] Therefore, many hydrophilic or macromolecule drugs (> 500 Da) cannot be penetrated through the skin without reducing barrier properties by disrupting the SC and altering the structure of lipid through the use of physical (device based) or chemical (formulation based) penetration enhancement techniques.[108,110,111] So, the development of an effective TDD through the skin, it is essential to carefully understand the properties of the excipients used in formulations. Nevertheless, many poorly water soluble drugs are targeted for TDD using chemical enhancers which causes toxicity, skin irritation and infections, burns and scars in their clinical application.[108,110,112] To overcome this limitation, ILs were efficient to improve the solubility of a poorly soluble drug to increase its TDD. The feasibility of ILs has been proven to increase the drug transport via the skin by the experimental data, as well as molecular dynamics. Lin GS et al. simulated the model membrane with amphiphilic 1-octyl-3- methylimidazolium-based IL by using molecular dynamics combined with empirical force fields and reported that the cationic head of IL inserts into a cell membrane of a model bacteria.[105] Hence, the membrane permeability of polar ammonia molecules is increased due to disruption of the structural integrity of cell membranes. The imidazolium-based hydrophilic IL can fluidize the cell membrane to generate routes for the transmission of molecules and causes membrane permeabilization.[113] Moreover, it has been established that ILs can be capable to extract the lipids from physiological structures of SC and enhanced the transport by creating channels for drug molecules.[108] Previously reported that IL-in-oil (IL/o) microemulsions (MEs) were used to enhance the solubility of a poorly soluble drug to improve its TDDs. Recently, deep eutectic solvents (DESs) were employed as a skin enhancer to increase the transdermal delivery of drugs as well as disrupt/neutralize biofilm-forming bacteria.[108] DESs are consist of a mixture of compounds which have a lower melting points than that of individual components. Mitragotri and co-workers have reported that choline and geranate (CAGE) known as DES, have a potent antibacterial activity and significantly enhanced the transdermal delivery of antibacterial drugs without causing notable toxicity to keratinocytes and mice.[108,114] They revealed that the transdermal delivery of hydrophilic drug molecules (i.e., mannitol and cefadroxil) and macromolecules (i.e., ovalbumin, bovine serum albumin, and insulin) were significantly improved by using CAGE as compared with commonly used chemical permeation enhancers like ethanol and transcutol. Moreover, TDD of various unlikely drug candidates for example protein molecules, acyclovir, and methotrexate, among others profited importantly from IL incorporation can open the approach for a huge number of alternative promising drug molecules.[114–116]

1.5.6. ILs are in microemulsions (MEs)

Microemulsions (MEs) are of great attention because of their remarkable properties like high ability to solubilize both polar and non-polar substances, poor interfacial tension, large interfacial region, spontaneous formation and good microstructures, which render them as remarkable possibilities for various applications.[115–121] MEs are the microheterogeneous systems consisting of two immiscible fluids and stabilized through an interfacial film of surfactants, often with co-surfactants.[121] In general they are classified into three different categories depending on their microstructure such as water-in-oil, oil-in-water, and bicontinuous, which is influenced by their physicochemical properties and the proportions of their ingredients.[117,121] In the last couple of years, an expanding number of studies dealt with MEs, in which an ionic liquid is utilized as the oil phase, the aqueous phase and the surfactant. It has been shown that water and oil are not really the necessary polar and non-polar components that add to the development of MEs yet can be alternated by targeted ILs.In addition, as a rule ILs are considered to be amphiphilic which aids the development of MEs in aqueous or non-aqueous phase because of their special surface activity.

1.5.6.1. Role of ILs in the preparation of MEs

Based on the role of ILs, MEs can be categories into three classifications.

Non-aqueous IL MEs: In the most MEs, water is consider an crucial component, though its use has a restrictive effect on their applications because water is comparatively narrow liquid range of temperature, further due to slightly solubility or unstability of many compounds in water. Recently, it has been reported that some ILs are used as the replacement of water due to their polar phase properties in MEs. Generally, an organic apolar solvent, an IL, and a surfactant are required to form non-aqueous IL based MEs. The main advantage of non-aqueous MEs is in their stability over a more extensive scope of temperatures compared with water. Zech and coworkers examined the thermal stability of $[C_{16}mim][Cl]$+ decanol/EAN/dodecane and revealed that it was stable with temperature from 30 to 150^0C.[122] Table 1.2 shows different types of non-aqueous IL based MEs. The hydrophilic imidazolium based IL, 1-Butyl-3-methylimidazolium tetrafluoroborate ($[C_4mim]BF_4$) is most often used for the development of IL based non-aqueous MEs which can be ascribed to its wide convenience, low cost and moderately simple preparation.[121,123] Han et al. first report the non-aqueous ME containing $[C_4mim][BF_4]$ as the polar phase in 2004.[124]

Gao et al. also developed the non-aqueous IL based MEs where $[C_4mim]BF_4$ was used as the polar phase and the ME was described by using a phase diagram, DLS measurement, conductivity measurement and freeze-fracture transmission electron microscopy (FF-TEM) measurements.[123] Afterward, Chakrabarty et al. describe the impact of restriction on solvation and rotational relaxation of a probe molecule in this MEs.[125] The role of diverse organic solvents like toluene, p-xylene, benzene has been investigated in this microemulsion.[121] For the preparation of this ME, Ghosh and co-workers have used an IL with the variety of alkyl chain length and made out of TX-100, cyclohexane and have revealed that the long alkyl chain of octayl sulfate permits the anion to align itself alongside the TX-100 surfactant which enhances the inflexibility of the ME.[126] In addition, the IL of sulfate anion can create a stable ME in the existence of surfactants. As $[C_2mim][C_4SO_4]$ IL can be utilized as a polar area in the ME when two non-ionic surfactants, sorbitan laurate (Span-20) and polyoxoethylene sorbitan monooleate (Tween-80) are mixed in isopropyl myristate (IPM).[127] Therefore, there are various surfactants such as cationic, anionic and non-ionic are stated in the literature, which are used in MEs and various kinds of ILs like hydrophilic, hydrophobic, protic are used as polar phases for the formation of IL-based non-aqueous ME.[119–121,126] These IL-based MEs are used in reaction media for kinetic studies and for the synthesis of monodispersed palladium as well as silver nanoparticle.[116,125]

Table 1.2: IL-based non-aqueous MEs. Table reproduced with permission from ref. [121]

IL	Oil	Surfactant/co-surfactant	ME type
$[C_2mim][C_nSO_4]$, n = 2 or 6	Toluene	CTAB/pentanol	IL/O, O/IL
$[C_4mim][BF_4]$	Cyclohexane	TX-100	IL/O, BC, O/IL
$[C_4mim][BF_4]$	Cyclohexane	TX-100/ $[C_6mim][HSO_4]$	IL/O
$[C_4mim][BF_4]$	Toluene	TX-100	IL/O, BC, O/IL
$[C_4mim][BF_4]$	Cyclohexane, toluene	TX-100	IL/O
$[C_4mim][BF_4]$	toluene, benzene, *p*-xylene	TX-100	IL/O, BC
$[C_4mim][BF_4]$	Benzene	TX-100	IL/O
$[C_4mim][BF_4]$	Cyclohexane	TX-100/ethanol	IL/O

$[C_4mim][BF_4]$	Toluene	TX-100/ethanol	IL/O, BC, O/IL
$[C_2mim][NTf_2]$	Cyclohexane	TX-100	IL/O, BC, O/IL
$[N_{3111}][NTf_2]$	Cyclohexane	TX-100	IL/O, BC, O/IL
$[C_4mim][PF_6]$	Toluene	TX-100	IL/O, BC, O/IL
$[C_4mim][PF_6]$	Toluene	Tween-80	IL/O, BC, O/IL
$[C_4mim][PF_6]$	Ethylene glycol	TX-100	IL/O, BC, O/IL
$[C_2mim][C_2SO_4]$	Toluene	$[C_4mim][C_8SO_4]$	IL/O, BC, O/IL
$[C_2mim][C_2SO_4]$	Toluene	$[C_4mim][C_{12}SO_4]$	IL/O, BC, O/IL
$[Py][NTF_2]$	Benzene	$[C_4mim][AOT]$	IL/O
EAN	Dodecane	$[C_{16}mim][Cl]$	IL/O

Aqueous IL MEs: In industrial applications, water in IL MEs has a great attraction compared with other MEs because IL and water both are reported as green solvents where ILs are used as a non-polar solvent. 1-Butyl-3-methyl imidazoliumhexafluorophosphate ($[C_4mim]$ PF_6) is most often used in the MEs. An aqueous IL based ME comprised of TX-100, water and $[C_4mim]$ PF_6 was firstly reported by Gao et al.[123] DLS measurements and cyclic voltametry (CV) were used to confirm the different sub-regions of the MEs. The hydrodynamic radius of this MEs is enhanced with increasing quantity of ILs because of the swelling behavior of the MEs. Pandey and coworkers have used a cobalt (II) salt to investigate the MEs.[128] In recent, Rai and coworkers have prepared aqueous IL MEs by using the nonionic surfactant, polyoxoethylene lauryl ether (Brij-35).[129] Then the system was investigated by using SAXS and DLS measurements. The existence of free and bound water molecules into the aggregates is indicated by using FTIR absorption data. Aqueous IL MEs have broad application in the synthesis of nanoparticle. A successful extraction of hemoglobin from human blood has been reported by using the water/TX-100/$[C_4mim]PF_6$ system. Additionally, it also be utilized in enzymatic reactions as reaction media.[121] Nanda et al. have prepared a water in IL ME by using an IL, dibutylimidazolium bis(trifluoromethanesulfonylimide) ($[C_4C_4mim][Tf_2N]$) and characterized this system to measure pH and microviscosity of the medium through ^1H NMR.[130] Rai et al. have utilized a zwitterionic surfactant, N-dodecyl-N,Ndimethyl- 3-ammonio-1-propanesulfonate (SB-12) instead of non-ionic surfactants to define the w/IL ME.[121] Conversely, to build the uptake efficacy of surfactants and water in ILs, ethanol is required and they have described the quaternary system, by using DLS, FTIR, NMR, and UV–VIS measurements. Furthermore, the stability of MEs composed of $[C_4mim]$ PF_6 IL is increased by blending of a non-ionic surfactant (TX-100) with an anionic surfactant(AOT).[121]

Microemulsions with IL as a surfactant: Recently, using SAILs MEs developed the consideration compared to other two kinds of MEs. The surface active nature containing ILs is term as a SAIL and they have positive advantages over the conventional surfactants as far as development of a stable interface because of the promising interactions between the anion and cation.[38,121,131–134] In the literature, it has been well reported that water in oil MEs with the AOT surfactant.[135] Nevertheless, it is very unusual for the formation of IL/oil MEs using the AOT surfactant. The formation of w/o microemulsions using the AOT surfactant is facile because of the promising interaction between the water molecules and the inorganic cation of AOT (Na+, NH4 + etc.). When water is replaced by the IL, this kind of interaction does not exist.[119,135] Therefore, when the inorganic cation containing AOT surfactant is alternated by an organic cation then the formation of IL in oil (IL/o)ME could be possible. Utilizing this idea, Sarkar et al. used an anion exchange reaction to synthesize [C4mim][AOT] by combination of NaAOT and 1-butyl-3- methylimidazolium bromide [C4mim]Br and they have succeeded in formulating IL/o ME using [C4mim][AOT] as the surfactant, various types of IL as polar solvents where benzene as the non-polar solvent.[126] Then the DLS measurement, phase diagram, and emission spectra of Coumarin- 480 were used to characterize the ternary formulate ME system. Rao et al further reported that the uptake efficacy of double chained SAIL is improved with increasing the chain length of IL.[126,133] The loading capacity of IL is also expanded with reduce in the anion to cation interaction strength of added ILs and follows the order $[C_4mim][TF_2N]$, $[C_4mim][PF_6]$ and $[C_4mim][BF_4]$. Consequently, they have established that the quantity of the IL in the center of ME can easily controlled by varying the behavior of the IL.[132] One of the most favorable advantages of IL/o ME is their high thermal stability at the long range of temperature.[119] Sarkar et al. have prepared high temperature stable ME by using a SAIL of N,N-dimethylethanolammonium 1,4-bis(2-ethylhexyl) sulfosuccinate (DAAOT) where IL as polar media and IPM as non-polar solvent.[38] In addition, Banerjee and coworkers have prepared triple chain SAIL, [BHD][AOT] by using the anion exchange reaction between benzyl-nhexadecyldimethylammonium chloride (BHDC) and Na-AOT.[132] The synthesized two triple chain containing SAILs effectively created IL an based ME in IPM and two hydrophobic ILs, N-methyl-N-propylpyrrolidinium bis(trifluoromethanesulfonyl) imide ($[P_{13}][Tf_2N]$) and N,N,Ntrimethyl- N-propylammonium bis-(trifluoromethanesulfonyl) imide ($[N_{3111}][TF_2N]$). Moreover, these SAILs also create a large size of unilamellar vesicles (LUVs) in aqueous media with non-polar solvents.[119] It has also reported AOT free SAIL based MEs in literature. Kunz et al prepare MEs using a SAIL based surfactant, 1-hexadecyl-3-methylimidazolium chloride ($[C_{16}mim]Cl$), cosurfactant decanol, RTILs (ethyl-ammonium

nitrate (EAN) and [Bmim]- BF4) as polar media and dodecane as nonpolar media.[122] The formation of spherical aggregate is proved by using the SAXS mesurement. The rigidity of the MEs changes with altering of the IL from EAN to [Bmim]-BF4. Like cationic SAILs, a long chain containing anion based SAILs could also be used to formulate IL MEs. A recent study showed that the IL/o MEs can be prepared by using SAIL of 1-butyl-3-methylimidazolium dodecyl sulfate ([Bmim][$DodSO_4$]), 1-ethyl-3-methylimidazolium ethylsulfate ([Emim][$EtSO_4$]) as polar solvents and toluene as non-polar solvent.[136] The utilization of polar solvents as [Emim][$EtSO_4$] has certain points of interest over various ILs because of the low toxicity, the relative wide window of fluidity, and low melting temperature. The swelling nature of MEs with gradual addition of IL is proved by using SAXS and DLS experiments.[119] Hence, these recently prepared SAILs have certain advantages over the other conventional surfactants and the surface-active nature of this kind of SAILs can be tuned relying on various conditions.

1.5.6.2. IL-based MEs in drug delivery

In recent, the MEs are of extensive attention to the pharmacological area, because of their capability to perform as a drug delivery vehicle as well as more beneficial like bio-compatibility, long-term stability, nanometer-sized aggregations as well as straightforward preparation.[121] The extensive solubilizing ability of ILs in mixture with the high permeability of oils, which has prompted to the effective use of IL/o MEs as transdermal drug delivery vehicles.[137] Recently, it has been developed the transdermal delivery of the hemostatic drug Dencichine using an imidazolium IL-based MEs.[138] Interestingly, in a study on IL and DES based-ME systems to the transdermal delivery of the antimalarial drug, artemisinin (ART). The formulation consisted of the DES (lidocainium ibuprofenate) and IL (1-hydroxyethyl-3-methylimidazolium chloride) as the oil phase and an internal aqueous phase as well as skin enhancer, respectively while Tween 80/span 20 (1:1) and ethanol were used as the surfactant and the co-surfactant, respectively. However, the prepared MEs were significantly enhanced the transport of dencichine by 10-fold higher and showed noteworthy higher hemostatic activity of drug than that of the drug aqueous solution during *in vivo* experiment. Goto and co-workers are the pioneers of this concept and reported that IL-based ME systems (IL-in-oil microemulsions) in which the drugs loaded-IL (drugs loaded in the IL core) is dispersed in oil in the existence of a surfactant (Figure 1.8).[109,116,117] They have developed that the solubility of acyclovir was increased 500 times more in dimethylimidazolium dimethylphosphate IL and increased 6 times higher the transdermal delivery than that of commercial acyclovir creams.

Recently, they also reported a solid-in-oil nanodispersion by using 1-dodecyl-3-methylimidazolium-based IL to deliver ovalbumin in vitro.[139]

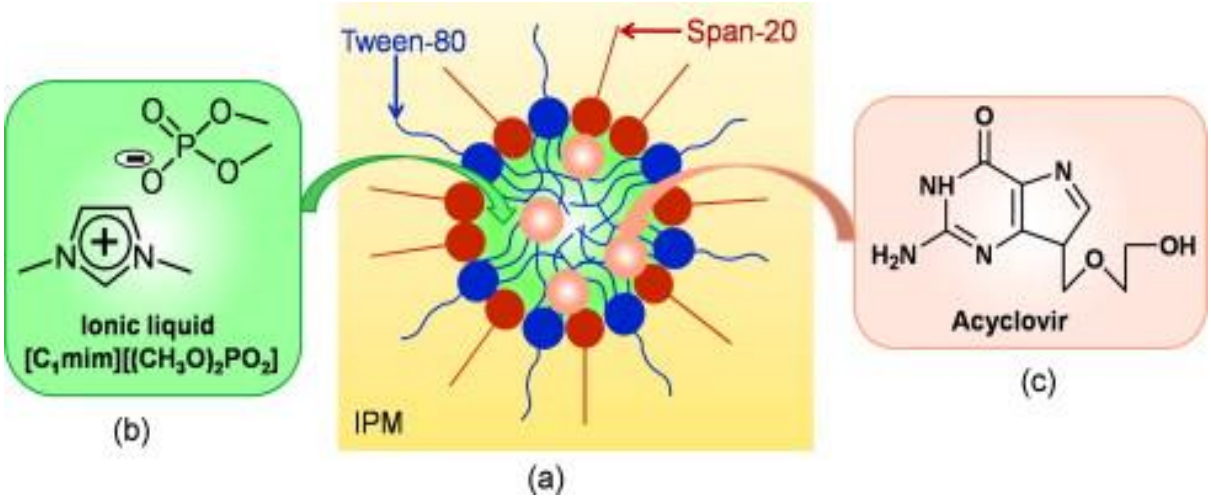

Figure 1.8. (a) Schematic illustration of IL/o MEs containing drug molecules. Chemical structure of IL (b) and acyclovir (c). Figure reproduced with permission from ref. [116]

It has been established the greater capability of IL-based MEs in skin cancer treatment compared to commercially available DDS. An effective drug 5-fluorouracil (5-FU) which currently available in ointment form reveals the low skin permeation efficacy and disagreeable dermal side effects which cause to non-compliance of patients in treatment.[121] Moreover, the unwanted skin reactions, like erythema and pain were avoided because of the drugs are encapsulated in the internal core of the ME. An IL/W ME was reported in where ex-vivo permeation studies of a poorly water soluble drug, etodolac was conducted for the topical delivery using 1-butyl-3- methylimidazolium hexafluorophosphate and demonstrated more efficient to transport the drugs by 2.1- and 1.6-fold higher than oily solution and O/W ME, respectively.[121] Moreover, histopathological studies also proven that the system did not cause any anatomical or pathological deviations in the skin. Another possible application of IL as a surfactant in which successfully replaced or combined with conventional surfactants. Imidazolium based ILs containing long alkyl chain were selected mostly because of their surface active properties in the W/o ME stabilization.[140,141] Zhou et al. have also developed the effective as well as benign synthesis of starch nanoparticles in [C_4mim][PF_6]/TX-100/1-butanol/H_2O.[142] Despite having considerable advantages, the use of ILs with ammonium, imidazolium, pyridinium, morpholinium and quinolinium cations is limited in clinical applications because of their high toxicity and low biocompatibility. Interestingly, ILs containing choline and organic acid (e.g., amino acid, fatty acid, and carboxylic acid) are considered to be non-toxic, biocompatible, and biodegradable as well as multi-functional

properties because of their biological sources. Cholinium geranate was significantly improved the transport of hydrophilic drug molecules (i.e., mannitol, cefadroxil and nobiletin) and macromolecules (i.e., bovine serum albumin, ovalbumin, RNA and insulin) when compared with conventional chemical permeation enhancers such as ethanol and Transcutol.[142] Therefore, IL-based MEs have been successfully conducted in a broad range of applications.

1.6. Aim and outline of this thesis

In the last few years, it has been widely reported that ILs are considered to be a superior solvent, remarkable surface active components, which are emerging as novel media in pharmaceuticals applications because of their tunable physical, chemical, and biological properties. Moreover, the multifunctionality of ILs can give an exceptional chance to design a definite process which might potentially decrease the process cost and an important effect on environmental factors. The current work mainly targeted on synthesis and characterization of new biocompatible surface active ionic liquids and investigated its biomedical applications. A review of the literature demonstrated that ILs are used in various applications in different areas like electrochemistry, catalysis, lubrication and solvents for synthetic chemistry yet there are a limited number of publications on the drug delivery. Therefore, ILs have huge potential to be discovered in these fields due to their exceptional properties. Thus the target of this research was the study of some biocompatible IL-based surfactants for the use in an effective drug delivery.

The overall objectives of the current work were shown as below:

A. Synthesis a series of potent biocompatible surface-active ionic liquids (SAILs) such as cholinium oleate, cholinium linoleate and cholinium erucate.

B. Study on the physico-chemical and thermal properties of the newly synthetized SAILs.

C. Determination of critical micelle concentration (CMC) of SAILs.

D. Study on surface active properties and micellization behavior of SAILs.

E. Study on *in vitro* cellular viability as well as the cytotoxicity of the SAILs on cell line NIH3T3.

F. Study on the biomedical application of the SAILs.

The specific objectives in each chapter are defined as follows:

Chapter 2 – A series of surface-active ionic liquids (SAILs) were obtained from natural fatty acids like oleic acid, linoleic acid, erucic acid and introduced as a biocompatible cation cholinium. The synthesized SAILs were characterized by using FTIR, ^{1}H and ^{13}C NMR, differential scanning calorimetric and elemental analyses. The CMC, surface activity, micellar properties and cytotoxicity SAILs were evaluated for envisaging their biomedical applications.

Chapter 3 – The main objective in this chapter was to develop a cholinium fatty acid IL-assisted non-aqueous ME by replacing Tween-80 and thus to introduce a promising alternative to conventional surfactants. The MEs were prepared by using SAILs as the surfactant, span 20 as the cosurfactant, isopropyl myristate (IPM) as the continuous oil phase and IL1,3-dimethylimidazoliumdimethyl phosphate as the non-aqueous polar phase. The prepared MEs were characterized by phase behavior study as well as dynamic light scattering (DLS) measurement. Then the *in vitro* cytotoxicity of the certain MEs was observed by using a three-dimensional reassembled human epidermis model. Finally, the drugs encapsulation efficiency and stability of drug loaded MEs are investigated for envisaging their pharmaceutical application as a biocompatible component of DDS.

Chapter 4– We summarized the findings of this research and briefly discussed further research directions related to this research.

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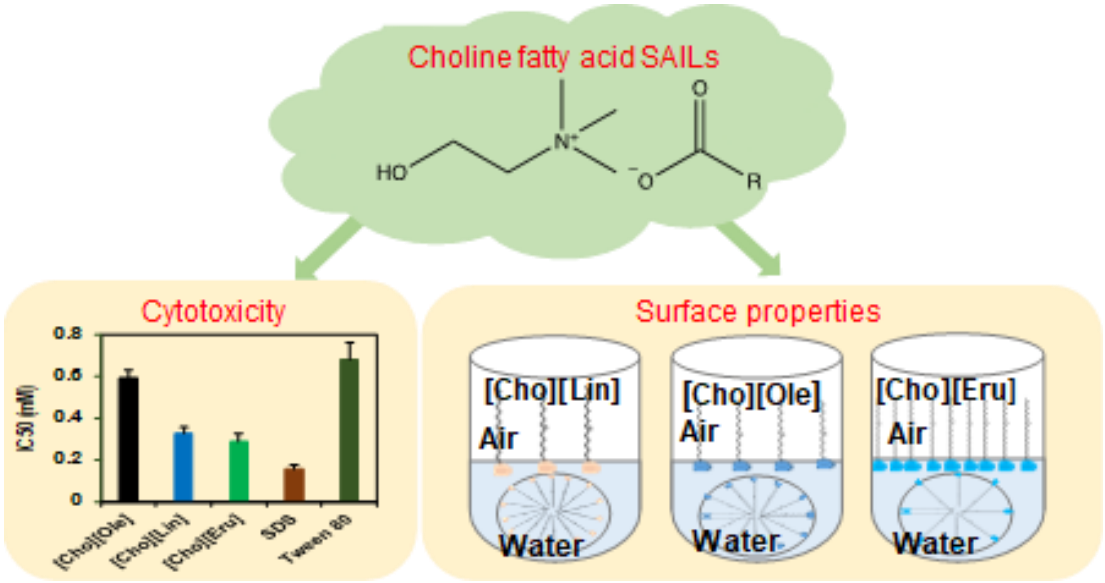
CHAPTER 2: SYNTHESIS AND CHARACTERIZATION OF CHOLINE–FATTY-ACID-BASED IONIC LIQUIDS: A NEW BIOCOMPATIBLE SURFACTANT



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2.1. Abstract

Ionic liquid (IL) surfactants have attracted great interest as promising substitutes for conventional surfactants owing to their exceptional and favorable physico-chemical properties. However, most IL surfactants are not eco-friendly and form unstable micelles, even when using a high concentration of the surfactant. In this study, we prepared a series of halogen-free and biocompatible choline–fatty-acid-based ILs with different chain lengths and degrees of saturation, and we then investigated their micellar properties in aqueous solutions. Characterization of the synthesized surface-active ILs (SAILs) was performed by ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy, Fourier transform infrared spectroscopy, differential scanning calorimetry, and elemental analysis. The surface-active properties of the SAILs were investigated by tensiometry, conductometry, and dynamic light scattering measurements. The critical micelle concentration of the SAILs was found to be 2 to 4 times lower than those of conventional surfactants. The thermodynamic properties of micellization (ΔG^0_m, ΔH^0_m, and ΔS^0_m) indicate that the micellization process of the SAILs is spontaneous, stable, and entropy-driven at room temperature. The cytotoxicity of the SAILs was evaluated using mammalian cell line NIH 3T3. Importantly, [Cho][Ole] shows lower toxicity than the analogous ILs with conventional surfactants. These results clearly suggest that these environmentally friendly SAILs can be used as a potential alternative to conventional ILs for various purposes, including biological applications.

2.2. Introduction

Surfactant-based technologies are extensively used in many industries, including the textile and leather, food, cosmetic, pharmaceutical, and detergent industries.[1–3] However, many of conventional surfactants particularly anionic and cationic surfactants, such as SDS, SDBS, cetyl trimethyl ammonium bromide, etc are toxic and non-biodegradable.[4–6] In addition, many surfactants require a large concentration to form stable micelles in aqueous media.[7] To address these issues, researchers are attempting to improve the surface-active properties by varying the physico-chemical conditions. Recently, many surface-active ionic liquids (SAILs) such as choline or amino acid-based ionic liquids have attracted considerable attention as environmentally friendly surfactants[6,8], they are promising substitutes for conventional surfactants because of their exceptional and favorable properties, such as their high thermal stability, negligible vapor pressure, biodegradability, non-volatility, and non-flammability.[6,9,10] The tailor-made properties of SAILs are important properties to obtain good surface activity in aqueous suspensions or emulsion formulations, which is not possible for conventional

surfactants.[7,11] SAILs are capable of decreasing the interfacial tension between polar and nonpolar solvents in water, even in high salt concentration solutions at room temperature.[7,12] ILs with a long-chain tail attached to the cation form self-assembled aggregates (such as micelles) in aqueous and mixed solutions.[13,14] Even though a variety of SAILs based on unlike cations, including imidazolium, pyridinium, pyrrolidinium, and morpholinium, have been synthesized, they are harmful to the environment because of their high toxicity and non-biodegradable nature.[9,15,16]

Amino acids and choline-based IL surfactants have attracted much attention for preparation of biocompatible SAILs because of their environmentally friendly properties, such as low toxicity and high biodegradability.[9,15,17] However, in most cases, synthetic SAILs contain quaternary nitrogen as the cation (e.g., imidazolium, pyrrolidinium, and alkylammonium) and a halogen atom as the anion (e.g., Br, F, and Cl), which can be released as corrosive HCl or HF during their undesirable hydrolysis under certain conditions.[9,18,19] They cause serious water pollution by release through wastewater effluents.[1] Considering these facts, environmentally friendly SAILs without halide ions are highly demanded for formation of surface-active vesicles in many processes, such as separation and adsorption, demulsification, and pretreatment of biomass.[7] Many researchers have reported biodegradable IL surfactants using a counteranion (e.g., carboxylate and alkyl sulfate) and a bio-based countercation (e.g., amino acid- and choline-based ions).[19–21] These IL surfactants satisfy the low toxicity requirement for efficient bio-friendly applications. Recently, Singh et al.[6] reported an eco-friendly and biodegradable choline-based surfactant (cholinium dodecylbenzenesulfonate) produced with a commercial surfactant (sodium dodecylbenzenesulfonate) and demonstrated its nontoxic nature using a freshwater microalgae *Scenedesmus* sp. D. Rengstl et al.[21] also reported low toxic and biocompatible choline carboxylates based ionic liquids and evaluated their biological membrane interaction with the liposomes. In addition, Trivedi et al.[9] prepared task-specific biodegradable amino-acid-based SAILs with better surface activity than conventional surfactants and revealed their potential application for reducing harmful algae blooms in sea water.

In this study, we synthesized and characterized three halogen-free choline–fatty-acid-based IL surfactants with different chain lengths and degrees of saturation: choline oleate ([Cho][Ole]), choline linoleate ([Cho][Lin]), and choline erucate ([Cho][Eru]) (Figure 2.1A–C, respectively). The purities and molecular structures of the synthesized ILs were evaluated by nuclear magnetic resonance (NMR), Fourier transform infrared (FT-IR) spectroscopy, and elemental

analysis. Conductivity and surface tension measurements were performed to determine their critical micelle concentrations and aggregation behavior in aqueous solution. The interfacial properties, such as the surface excess concentration, minimum surface area per molecule, effectiveness, and efficiency, were also determined from the equilibrium surface tension data. Finally, the toxicological effects of the SAILs were evaluated using the NIH 3T3 cell line.

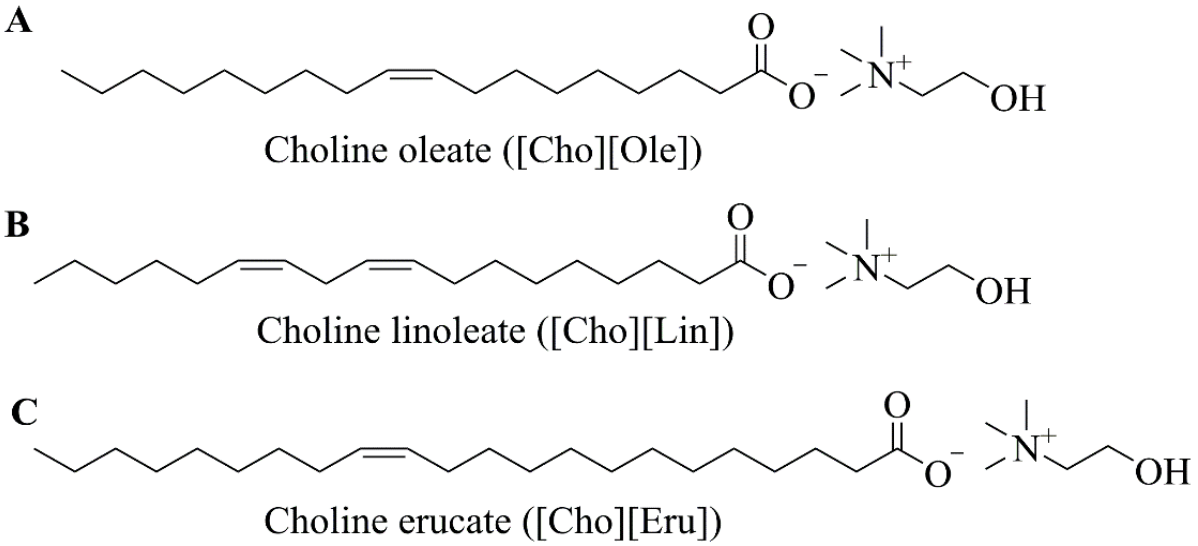


Figure 2.1. Structures of the choline-based IL surfactants. (A) choline oleate, (B) choline linoleate, and (C) choline erucate.

2.3. Materials and methods

2.3.1. Materials

Oleic acid, linoleic acid, and erucic acid (anhydrous, >99% purity) were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Methanol, ethanol, isopropanol, dimethyl sulfoxide (DMSO), hexane, toluene, ethyl acetate, chloroform, acetone, choline chloride, minimal essential medium (MEM), Opti-MEM, fetal bovine serum, and antibiotic–antimycotic were also obtained from Wako Pure Chemicals Industries.

The WST-8 cell counting kit was purchased from Dojindo Molecular Technologies, Inc. (Kumamoto, Japan). NIH 3T3 was obtained from the RIKEN cell bank (Tsukuba, Japan).

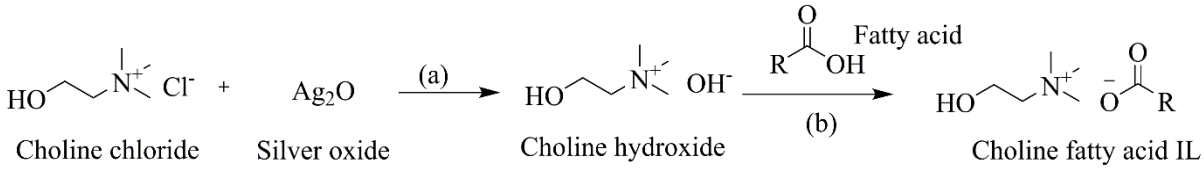
2.3.2. Synthesis of choline hydroxide for the SAILs

The choline cation was synthesized according to our established procedure.[22] In brief, 5.0 g of choline chloride was dissolved in water and vigorously stirred. The solution was then stirred with an excess of silver oxide and allowed to stand for 2 h in the dark at room temperature

(Scheme 2.1a). The resultant solution was then filtered and evaporated using reduced pressure to obtain choline hydroxide. The concentration of choline hydroxide was determined by Mitsubishi Karl Fischer titration (Tokyo, Japan).

2.3.3. General synthetic procedure of the SAILs

The choline–fatty-acid-based IL surfactants were synthesized according to a previously reported protocol.[7,8] In brief, an equimolar mixture of the fatty acid and choline hydroxide in methanol was thoroughly stirred and then allowed to stand for 24 h at room temperature (Scheme 2.1b). After neutralization, the resultant solution was evaporated at 40^0C temperature and 20 hPa pressure, and then freeze-dried for 48 h to remove the remaining water. The purities and molecular structures of the synthesized products were characterized by ^{1}H and ^{13}C NMR and elemental analysis.



Scheme 2.1. General process for synthesis of the SAILs. (a) Precipitation of silver chloride in water at room temperature for 2 h in the dark to obtain choline hydroxide. (b) Thorough stirring of choline hydroxide and the fatty acid in methanol at room temperature for 24 h to obtain the choline–fatty acid IL.

2.3.4. NMR measurements

The ^{1}H and ^{13}C NMR spectra of the SAILs were recorded in deuterated DMSO or methanol with tetramethylsilane as the internal standard using a JEOL ECZ400S 400 MHz spectrometer (Tokyo, Japan) operated at 25 °C. The unit of the coupling constants (*J*) is hertz (Hz). The DeltaV software package (version 5.0.5.1, JEOL) was used to process the spectra.

The purities of the SAILs were calculated using the following equation:

$$\text{Purity (\%)} = [\Sigma I\,(\text{product})/\Sigma I\,(\text{total})] \times 100 \quad \ldots\ldots (2.1)$$

where *I* is the relative area of each signal.[23,24]

Characterization of Choline oleate

Yield 91.1%. Purity 99.2%. Highly viscous liquid. ^{1}H-NMR (DMSO-D6, 400 MHz, TMS) δ ppm: 0.91-0.88 (m, 3H), 1.32-1.29 (m, 21H), 1.59 (t, J = 7.1 Hz, 2H), 2.03 (q, J = 6.3 Hz, 4H),

2.20 (t, J = 7.5 Hz, 2H), 3.31-3.21 (m, 9H), 3.49-3.47 (m, 1H), 4.01-3.98 (m, 1H), 5.34 (t, J = 5.3 Hz, 2H). ^{13}C-NMR (CDCl3, 400 MHz, TMS) δ ppm: 13.11, 21.68, 25.79, 26.24, 26.29, 27.98, 28.32, 28.34, 28.51, 28.55, 28.68, 28.78, 28.91, 28.95, 30.79, 30.90, 37.33, 53.21, 54.86, 67.00, 75.81, 76.13, 76.34, 76.45, 128.74, 128.91, 179.22.

Characterization of Choline linoleate

Yield 90.18%. Purity 99.5%. Highly viscous liquid. ^{1}H-NMR (DMSO-D6, 400 MHz, TMS) δ ppm: 0.86 (dd, J = 8.0, 5.7 Hz, 3H), 1.43-1.22 (m, 16H), 1.83 (t, J = 7.3 Hz, 2H), 2.04-1.97 (m, 4H), 2.73 (t, J = 6.4 Hz, 2H), 3.19-3.12 (m, 9H), 3.47-3.44 (m, 2H), 3.88-3.84 (m, 2H), 5.38-5.26 (m, 4H). ^{13}C-NMR (CDCl3, 400 MHz, TMS) δ ppm: 13.23, 22.35, 25.26, 26.51, 26.87, 26.94, 29.12, 29.19, 29.29, 29.53, 29.59, 31.37, 37.95, 47.08, 47.29, 47.51, 47.73, 47.94, 48.15, 48.36, 53.33, 53.37, 53.41, 55.74, 67.72, 127.74, 127.76, 127.92, 129.63, 181.37.

Characterization of Choline erucate

Yield 87.13%. Purity 99.0%. Highly viscous liquid. ^{1}H-NMR (DMSO-D6, 400 MHz, TMS) δ ppm: 0.90 (t, J = 6.9 Hz, 3H), 1.29 (s, 28H), 1.59 (d, J = 14.6 Hz, 2H), 2.16-2.00 (m, 6H), 3.20 (s, 9H), 3.48 (t, J = 4.8 Hz, 2H), 3.99 (dt, J = 9.9, 2.7 Hz, 2H), 5.34 (t, J = 4.6 Hz, 2H). ^{13}C-NMR (CDCl3, 400 MHz, TMS) δ ppm: 14.15, 22.71, 26.98, 27.24, 27.28, 29.34, 29.43, 29.55, 29.71, 29.80, 29.86, 30.08, 31.93, 38.72, 54.32, 55.94, 68.20, 76.87, 77.18, 77.50, 129.90, 180.09.

2.3.5. Elemental analysis

The carbon, hydrogen, and nitrogen contents of the SAILs were determined with a Yanaco CHNC MT-5 analyzer (Kyoto, Japan).

Choline oleate: Found: C, 68.20; H, 12.14; N, 3.44%. Calc. for $C_{23}H_{47}NO_3.H_2O$: C, 68.37; H, 12.17; N, 3.47%.

Choline linoleate: Found: C, 68.20; H, 12.14; N, 3.44%. Calc. for $C_{23}H_{47}NO_3.H_2O$: C, 68.37; H, 12.17; N, 3.47%.

Choline erucate: Found: C, 68.20; H, 12.14; N, 3.44%. Calc. for $C_{23}H_{47}NO_3.H_2O$: C, 68.37; H, 12.17; N, 3.47%.

2.3.6. Fourier transform infrared analysis

The FT-IR spectra of the SAILs were recorded with a Perkin Elmer Frontier FT/IR spectrometer (Perkin Elmer, MA) containing a lessened whole reflection sampler equipped

with a diamond crystal. All of the spectra are the sum of 20 scans in the wavenumber range 400–4000 cm^{-1}.

2.3.7. Conductivity measurements

A seven compact conductometer (Mettler Toledo Instruments) was used to measure the conductivity of the SAILs at 25 °C with an uncertainty of ±0.01 mS cm^{-1}. The critical micelle concentration (CMC) of each SAIL was obtained using the position of the sharp break point in the linear dependency of the conductivity against the concentration. All of the SAIL solutions were diluted with Milli-Q water.

2.3.8. Surface tension measurements

The surface tension measurements were performed with a DropMaster DMo-501 analyzer at room temperature. The shape drop method was used to determine the surface tension. The principle of this method is formation of an axisymmetric drop at the tip of the needle of the syringe. The image of the drop was taken and digitized using a charge-coupled device camera. The surface tension (ϒ in mN/m) was calculated by the Laplace equation. During the measurement, a photo of the tested droplet was taken. All of the IL samples were prepared in MilliQ water by serial dilution.

2.3.9. Dynamic light scattering measurements

The dynamic light scattering (DLS) measurements were performed with a Zetasizer (Nano Series, Malvern Instruments, UK). The scattering angle was 173° and the measurements were performed at 25 ± 0.1 °C. A 1-cm-path-length quartz cuvette was used for the measurements. Before the measurements, the samples were filtered through a 0.45-μm filter. An average of 10 measurements was considered as the experimental data. The mean micelle size was calculated by Malvern DTS software.

2.3.10. Cytotoxicity evaluation

Viability evaluation of the WST-8 cell was performed using mouse fibroblast cell line NIH 3T3 by a previously reported method with some modifications.[23] The cells were cultivated on a cell culture dish and trypsinized to collect the cells from the dish. The target cells were matured at 5000 cells/well in a 96-well flat-bottomed plate system and cultured for 24 h in MEM (containing 10% fetal bovine serum and 1% antibiotic–antimycotic) at 37 °C in a CO_2 incubator. The SAIL, sodium dodecyl sulfate (SDS), and Tween 80 samples were prepared in Opti-MEM with concentrations in the range 0.001–40 mM. The medium of each well was then

replaced by 100 μL of each sample solution and the plates were kept in an incubator for 24 h. The wells were then washed twice with phosphate-buffered saline after the solution was removed from each well. The mitochondrial activities of the cells were measured using 100 μL of Opti-MEM containing 10 μL of the WST assay reagent. After 3 h incubation at 37 °C in a CO_2 incubator, the absorbance of the supernatant ($A_{treated}$) was measured at 450 nm with a microplate spectrophotometer (Bio-Rad, Tokyo, Japan). A control ($A_{control}$) was prepared by dilution of the cell in Opti-MEM without a sample. The relative cell viability indicates the cytotoxicity, which was determined by the following equation:[23]

$$\text{Cell viability (\%)} = (A_{treated}/A_{control}) \times 100 \quad (2.2)$$

The average values of three repeated measurements are reported. Microsoft Excel 2016 (Microsoft, Washington, USA) was used for statistical analysis of the data. The half maximal inhibitory concentration (IC_{50}) of each surfactant was calculated. The results are given as the mean and standard deviation.

2.3.11. Differential scanning calorimetry

The differential scanning calorimetry (DSC) measurements were performed with a Hitachi High-Technologies DSC X7000 calorimeter (Tokyo, Japan). Standard aluminum pans containing 4–6 mg samples were crimped with an aluminum lid using a press (T Zero sample press). The different SAILs surfactants were scanned in the temperature range between -50°C and 100°C at a heating rate of 2°C/min under constant nitrogen flow of 30 mL/min. Three cycles of each sample were measured. The reference was an empty aluminum pan.

2.4. Results and discussion

2.4.1. Synthesis and characterization of the SAILs

The choline–fatty-acid-based IL surfactants were synthesized by fully ionization of the fatty acid and choline hydroxide in methanol. When equimolar amounts of the fatty acid and choline hydroxide were added to methanol, a clear solution was obtained, which indicates completion of the reaction. After evaporation of methanol and freeze drying, the SAIL compounds appeared as highly viscous liquids. The yields of the synthesized SAILs were ≥90.0%. The purities and structures of all of the synthetic compounds were confirmed by ^{1}H and ^{13}C NMR, FT-IR spectroscopy, and elemental analysis. ^{1}H NMR confirms that the stoichiometry of the two constituents of the SAILs is 1:1 (Figure 2.2). The carboxylic proton of the fatty acid (–COOH peak at 11.88 ppm for linoleic acid in Fig. 2.2B) is not present in all of the SAIL spectra.

The absence of this peak confirms that all of the fatty acids and choline hydroxide transform to SAIL surfactants.

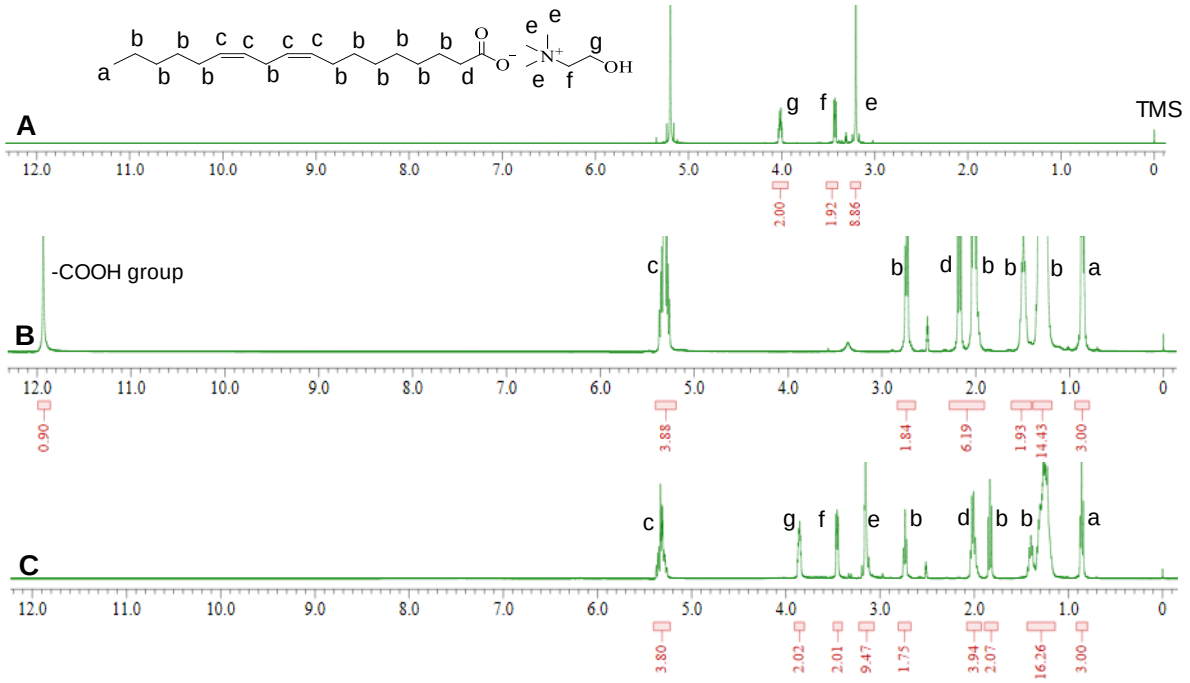


Figure 2.2. ^{1}H-NMR spectra of (A) choline, (B) free linoleic acid, and (C) the choline linoleate IL.

To investigate the structural changes of the SAILs, FT-IR spectroscopy was performed to assess ionization between the free fatty acid and choline cation. The FT-IR spectra of free erucic acid, choline hydroxide, and the [Cho][Eru] IL surfactant are shown in Figure 2.3. Broad and strong vibrational peaks for asymmetric and symmetric C–H stretching of the methylene and methyl units are observed at 2800–3000 cm^{-1} in both the spectra of the free acid and [Cho][Eru] IL. Further, vibrational peaks of the double bonds in the SAIL are observed at 2993–3012.5 and ~956.5 cm^{-1} owing to the =C−H stretching and =C−H bending modes, respectively. However, the characteristic peak of C=O stretching of the –COOH group observed at 1692 cm^{-1} in the erucic acid spectrum shifts to 1576 cm^{-1} for C=O stretching of the carboxylate after formation of the [Cho][Eru] IL.[25] The characteristic peak of the quaternary ammonium group at ca. 1638 cm^{-1} in the choline cation spectrum shifts to about 1623 cm^{-1} in the [Cho][Eru] IL spectrum (Figure 2.3). Similar characteristic peaks are observed in all of the SAIL spectra. For the [Cho][Eru] IL surfactant, the characteristic peak of C=O stretching of the –COOH groups at 1692 cm^{-1} for the free acid is not observed, which suggests a strong interaction between the –COOH groups of the free fatty acid and the quaternary ammonium groups of the cations.

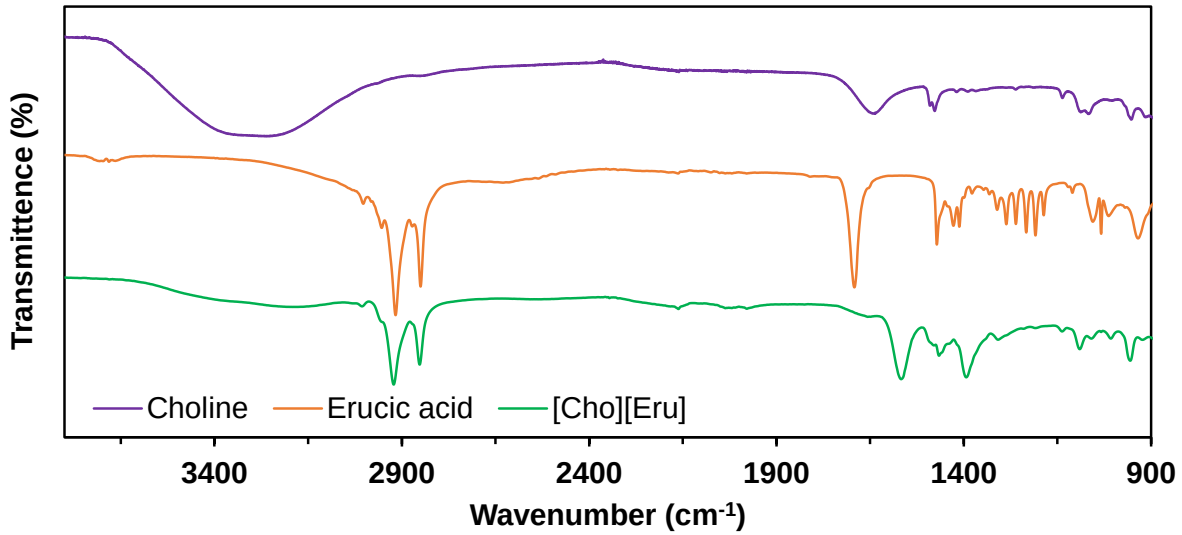


Figure 2.3. FT-IR spectra of choline hydroxide, free erucic acid, and the [Cho][Eru] IL.

DSC is a convenient thermal analysis technique to investigate the physical properties of SAIL surfactants, such as the melting point and/or phase transitions. It has been reported that the variation of melting temperatures and enthalpies between freshly melted and reheated samples were occurred due to the formation of polymorphic metastable phases.[26] In general, three cycles of samples are preferred, whereas the 2nd and 3rd cycles indicating reproducibility and validity of the measurements (non-leaking sample holder, temperature stability and nondegraded substance).[26,27] However, three cycles DSC thermograms of the [Cho][Ole] IL as a representative fatty acid IL surfactant are shown in Figure 2.4(A). During the heating run, the phase transitions observed with increasing the temperature, which can be classified as crystalline (Cr), semicrystalline (CrM) and liquid crystalline (LC). The first melting step of 2nd cycle was observed at around -9.5 °C due to the transitions from Cr phases to CrM phases that seems to be occurred for slightly molten of alkyl chains of [Cho][Ole] IL. The heat flow change of relaxation for this transition was -0.9 mW. The 2nd melting steps was at approximately 7.6 °C with heat flow change of -1.1 mW, which indicated the melting of CrM phases to LC phases because of the simultaneous melting of the polar groups and the partly molten alkyl chains of IL. Upon cooling run of each cycle, the [Cho][Ole] IL showed one sharp reproducible transition temperature at -5.6°C with relatively large heat flow change of 0.8 mW that attributed to the transition from LC phases to Cr phases. Therefore, these result demonstrated a high reproducibility at all cooling and after second heating thermograms, which are in good agreement with previous studies.[26–28] However, the temperature hysteresis of [Cho][Eru] IL is significantly differed due to the presence of long alkyl chain of fatty acid. The value of first temperature hysteresis has approximately 15.1 °C and 2nd around 35-45 °C (Figure 2.4(B)).

Because of the greater van der Waals interactions between increasing number of methylene units in the fatty acid anion, the melting temperature of the SAILs increases with increasing chain length.[26] The [Cho][Eru] IL surfactant has the highest melting temperature among the investigated SAILs because of the higher number of methylene units in the erucate anion (C_{22}) than in the oleate and linoleate anions (C_{18}). The [Cho][Lin] IL has a lower melting temperature (-38.1 °C) than the [Cho][Ole] IL because there are two double bonds in the linoleate anion, which decreases the van der Waals interaction.[29]

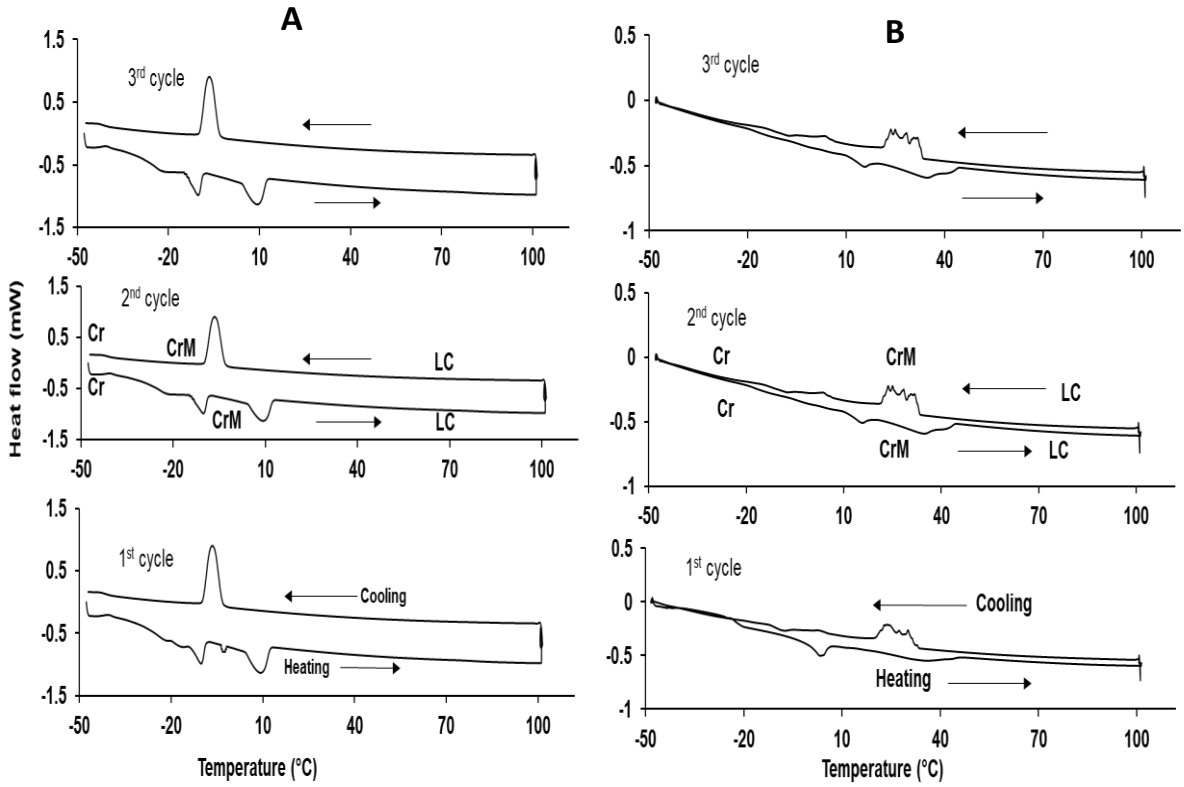


Figure 2.4. DSC thermograms of the (A)[Cho][Ole] ILs and (B)[Cho][Eru] ILs as representative choline-based IL surfactants.

2.4.2. Surface active properties and micellization parameters of the SAILs

The surface activities of the synthesized choline–fatty acid ILs were determined by surface tension measurements using a DropMaster DMo-501 analyzer at room temperature. The surface tension (γ) values of aqueous solutions of the ILs according to their concentrations are shown in Figure 2.5. As expected, the surface tension of the SAILs decreases with increasing concentration until the onset of the plateau region, which indicates the critical micelle concentration (CMC) of the respective SAIL.[30] The reason for the reduction of the surface tension in the pre-micellar region is adsorption of molecules to the hydrophobic portion of the

ILs at the air–liquid interface. The surface tension remains nearly constant with increasing IL concentration because of saturation of the air–liquid interface with IL molecules.

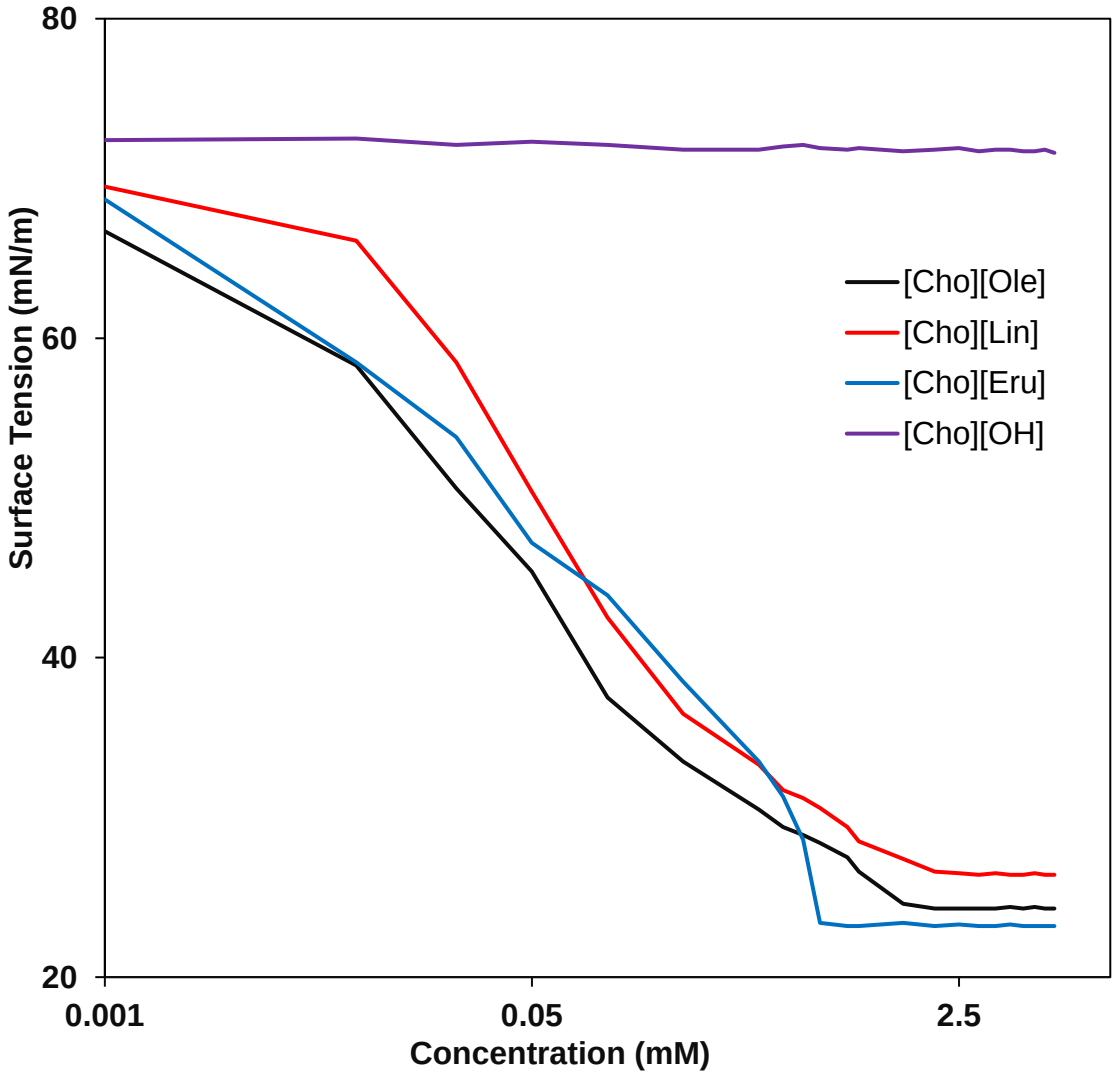


Figure 2.5. Variation of surface tension (γ) in aqueous solutions of different ILs as a function of their concentration at 25 °C.

The estimated CMC and surface tension at the CMC (γ_{CMC}) values of the SAILs are given in Table 2.1, which shows that the molecular structure of the anion has an extraordinary effect on the CMC value. The CMC values of the SAILs were following order: [Cho][Eru] < [Cho][Ole] < [Cho][Lin], which mainly depends on the chemical structure of the anion. Note that the surface tension of the choline cation with hydroxide anion is almost similar to the water. There are no significant changed the surface tension when the concentration of choline hydroxide was increased upto 10 mM (Figure 2.S2). However, the [Cho][Eru] surfactant has the lowest CMC value (0.8 mM) among the investigated SAILs and the conventional ionic surfactant SDS,

possibly because Eru is the most hydrophobic anion owing to it having the longest alkyl chain. The CMC and γ_{CMC} values of the SAILs in water are considerably lower than those of the conventional ionic surfactants (SDS and sodium dodecyl benzene sulfate (SDBS)), suggesting that the SAILs are highly effective surfactants.

To further understand the interfacial properties, the CMC values of the SAILs were used to calculate other parameters, such as the effectiveness of surface tension reduction (Π_{CMC}), adsorption efficiency (pC_{20}), surface excess concentration (Γ_{max}), and minimum surface area per molecule at the interface (A_{min}). Π_{CMC} and pC_{20} are two important parameters that reveal the surface activity of surfactant molecules in aqueous solution [31]. At the CMC, Π_{CMC} was determined by the following equation:[7]

$$\Pi_{CMC} = \gamma_o - \gamma_{CMC} \quad \text{(2.3)}$$

where γ_o and γ_{CMC} are the surface tensions of the pure solvent and solution at the CMC, respectively. As expected, the Π_{CMC} values of the SAILs are in the reverse order to γ_{CMC}. A higher Π_{CMC} value indicates a higher capability to decrease the surface tension of the aqueous system. The adsorption efficiency of surfactant molecules at the air–aqueous interface increases with increasing pC_{20}, which was calculated by the following equation:[32]

$$pC_{20} = -\log C_{20} \quad \text{(2.4)}$$

where C_{20} is the IL concentration that reduces the surface tension of pure water by 20 $mN \cdot m^{-1}$. As listed in Table 2.1, both Π_{CMC} and pC_{20} value of the [Cho][Eru] surfactant is higher than those of SDS and SDBS, which confirms its superior adsorption efficiency for decreasing the surface tension of water. This is probably because of the longer hydrophobic alkyl chain of [Cho][Eru] or size difference of the cations (Cho^+ and Na^+), which is less hydrated by water and the electrostatic repulsion between the charged head groups in the surface layer is stronger.[6,7,33] Because of adsorption of SAILs at the air–aqueous solution interface, the maximum surface excess of the surface-active component was determined by the following form of the Gibbs adsorption equation:[34]

$$\Gamma_{max} = -\frac{1}{nRT}\frac{\partial \gamma}{\partial \ln C} \quad \text{(2.5)}$$

where n is the number of species formed in solution by dissociation of the surface-active molecule, R is the gas constant (R = 8.314 J/mol/K), T is the absolute temperature in K, and C is the IL surfactant concentration in mol/L. Here, we use the value for n is equal to 2 based on

the published literatures, where it was reported that IL surfactants including imidazolium-based halide or aromatic ionic liquids are fully dissociated in water.[32,34] We assumed that our ILs surfactants follow the same trend. Further, to understand the packing density of surfactant molecules at the interface, A_{min} was determined from Γ_{max} using the following equation:[35]

$$A_{\min} = \frac{10^{18}}{\Gamma_{\max} N_A} \quad \text{..... (2.6)}$$

where N_A is Avogadro's number and A_{min} is in nm^2. The arrangement of surfactant molecules at the air–water interface is reflected by Γ_{max} and A_{min}, which were obtained from the Gibbs adsorption isotherm. The estimated Γ_{max} values of the SAILs (Table 2.1) are in the following order: [Cho][Eru] > [Cho][Ole] > [Cho][Lin] > [Cho][DBS]. The order of the pC_{20} values is the same. The Γ_{max} value of [Cho][Eru] is higher than those of the other SAILs and traditional ionic surfactants, suggesting more compact aggregation because of the presence of a more hydrophobic alkyl chain. In addition, the intermolecular hydrogen bonding interaction between the carboxylic group and the cholinium group can result in the surfactant molecules more densely assembling at the air–liquid interface. However, greater adsorption of more molecules at the air–liquid interface depends on a higher Γ_{max} value. As a result, A_{min} is smaller.

The A_{min} values of the SAILs are in the reverse order to Γ_{max} (Table 2.1). As the carbon chain length increases, the A_{min} value of the synthesized SAIL decreases (Table 2.1), indicating more compact aggregation and higher packing density at the air–liquid interface [36]. Compared with [Cho][DBS], the A_{min} value of [Cho][Eru] is lower, which can be mainly attributed to the size of the choline counterion (Eru or DBS is the counterion of choline cation). By replacing long chain Cho^+ with Na^+, the higher A_{min} value indicates looser packing of surfactant molecules at the air–water interface, which was reported in previous studies.[6,37]

Table 2.1. Surface properties of choline-based IL surfactants in aqueous solution at 25 °C.

Surfactants	cmc (mM)	γ_{cmc} (mN/m)	Γ_{max} (μmol/m^2)	A_{min} (Å^2)	pC_{20}	Π_{cmc} (mN/m)
SDS[38]	7.8	39.6	3.5	148	2.4	32.5
SDBS[6]	2.9	32.6	1.8	94.2	3.7	38.5
[Cho][DBS][6]	2.2	31.9	2.6	64.7	4.2	39.2
[Cho][Lin]	2.0	26.6	3.2	51.4	4.4	44.9
[Cho][Ole]	1.7	24.4	3.8	43.2	4.6	47.1
[Cho][Eru]	0.8	23.2	9.0	18.5	4.7	49.3

2.4.3. Conductivity measurements and thermodynamic parameters of micellar formation

Electrical conductivity measurements were performed to evaluate the micellar properties of the SAILs in aqueous solution at room temperature. There is an observable change in the slope of the curve of the specific conductivity versus molar concentration for all of the SAILs (Figure 2.6). The slope of the line decreases with increases molar concentration, and the CMC is assigned to the break point of the plot.[39] Above the CMC, the conductivity slope abruptly decreases because of effective charge reduction for binding of counterions to the micelles.

The CMC values of the SAILs determined from the electrical conductivity plots are given in Table 2.2, which are in good agreement with those from the tensiometry measurements in Table 2.1. The ratio of the slopes above (S_1) and below (S_2) the CMC represents the degree of counterion binding (β):[40]

$$\beta = 1 - {}^{S_2}/_{S_1} \quad \text{..... (2.7)}$$

The β values of the SAILs at 25 °C are given in Table 2.2. As expected, the [Cho][Eru] surfactant has the highest β value because of the more hydrophobic erucate anion. In general, the β value increases with increasing hydrophobicity or decreasing CMC value of the ionic surfactant, which indicates the affinity to accumulate at the micellar interface.

Different thermodynamic parameters of aggregation, such as the standard Gibbs free energy of micelle formation (ΔG^0_m) and molar free energy at maximum adsorption (G_{min}), were determined to more clearly understand the micellization process of the surfactants. G_{min} was calculated by the following equation:[7]

$$G_{\min} = N_A \gamma_{CMC} A_{\min} \quad \text{..... (2.8)}$$

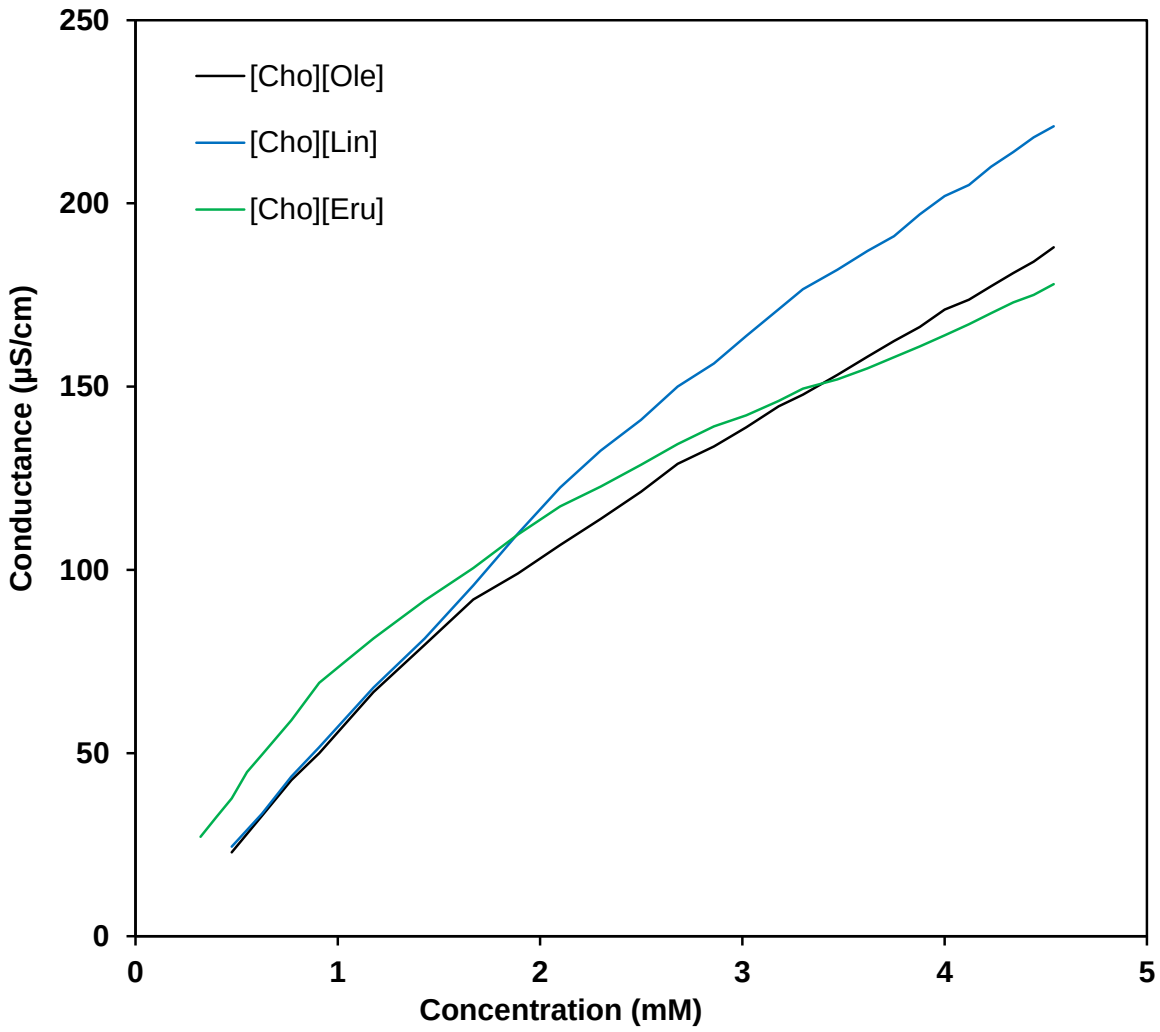


Figure 2.6. Plots of the electrical conductivity against the concentration for the SAIL surfactants at 25 °C.

The pseudo-phase model of micellization was used to calculate ΔG^0_m:[34]

$$\Delta G^0_m = RT(1+\beta)\ln X_{CMC} \quad \text{..... (2.9)}$$

where X_{CMC} is the molar fraction of the surfactant at the CMC ($X_{CMC} = CMC/55.4$, where the factor 55.4 is because 1 L of water contains 55.4 mol of water at 298 K). The ΔG^0_m values are given in Table 2.2. The ΔG^0_m values are negative and that of [Cho][Eru] is the most negative. This indicates that the micelle formation process of the [Cho][Eru] surfactant is a more spontaneous process than those of the other SAILs and the traditional surfactant SDBS. Moreover, ΔG^0_m decreases with increasing hydrophobicity of the anion, indicating that the micelle formation tendency increases with increasing hydrophobicity of the IL anion. The standard enthalpy of micellization (ΔH^0_m) and standard entropy of micellization (ΔS^0_m) were

calculated using the following equations obtained from the Gibbs–Helmholtz equation for aqueous solutions:[32]

$$\Delta H_{\mathrm{m}}^{0} = \frac{\partial(\Delta G_{\mathrm{m}}^{0}/T)}{\partial(\frac{1}{T})} \quad \text{(2.10)}$$

$$\Delta S_{\mathrm{m}}^{0} = (\Delta H_{\mathrm{m}}^{0} - \Delta G_{\mathrm{m}}^{0})/T \quad \text{(2.11)}$$

The negative ΔH^0_{m} values of all of the investigated SAILs (Table 2.2) indicate that micelle formation is exothermic. The main driving factor for micellization with a negative ΔH^0_{m} is considered to be the hydrophobic interaction between the surfactant and solvent.[32] ΔH^0_{m} is much smaller than $T\Delta S^0_{\mathrm{m}}$ (Table 2.2), which indicates that the micellization process is greatly affected by entropy gain. The large positive ΔS^0_{m} mainly contributes to the negative ΔG^0_{m}. Thus, micellization in aqueous solution is an entropy-driven process of transfer of the hydrophobic group from the solvent atmosphere to the inside of the micelle.

Table 2.2. Thermodynamic parameters of micelle formation for different SAILs at 25 °C.

Surfactants	cmc (mM)	β	ΔG^0_{m} (kJ/mol)	G_{min} (kJ/mol)	ΔH^0_{m} (kJ/mol)	$T\Delta S^0_{\mathrm{m}}$ (kJ/mol)	ΔG^0_{ad} (kJ/mol)
SDBS[6]	2.9	0.26	-30.2	18.1	-0.6	29.6	-52.0
[Cho][Lin]	2.1	0.35	-34.0	8.2	-2.4	31.6	-48.0
[Cho][Ole]	1.7	0.43	-36.8	6.3	-2.5	34.3	-49.2
[Cho][Eru]	0.9	0.57	-42.8	2.7	-2.4	40.4	-48.3

The standard Gibbs free energy of adsorption (ΔG^0_{ad}) at the air–liquid interface was calculated by the following equation [7]:

$$\Delta G_{\mathrm{ad}}^{0} = \Delta G_{\mathrm{m}}^{0} - \frac{\pi_{\mathrm{CMC}}}{\Gamma_{\mathrm{max}}} \quad \text{(2.12)}$$

The calculated ΔG^0_{ad} values of all of the SAILs are negative (Table 2.2), suggesting that adsorption of the surfactant molecules at the air–solution interface is a spontaneous process. The ΔG^0_{ad} values of the SAILs are more negative than the ΔG^0_{m} values, indicating that the adsorption process promotes micelle formation.

2.4.4. Sizes of micellar aggregations

DLS measurements were performed to investigate the particle sizes and size distributions of the micelles of the SAILs in aqueous solution. The investigated concentration of the SAIL

surfactants was kept at 8-10 times the CMC by dilution with water. The sizes and size distribution of micelles at 25 °C are shown in Figure 2.7(A). The [Cho][Ole] and [Cho][Lin] SAILs yield primary micelles 4.3 nm and 3.9 nm in diameter, respectively, which are similar to those reported with other ILs surfactants.[34,41] However, both SAILs produce agglomerated micelles due to the hydrophobic nature of long alkyl chain.[34,42] In case of [Cho][Eru], also two size distributions were obtained at 5.6 nm and 28.3 nm, in where the maximum scattering intensity was obtained at 28.3 nm because of the existence of highly hydrophobic alkyl chains in the aqueous phase. Volume-weighted size distributions of SAIL surfactants also showed that the volume of small micelles predominant against micellar agglomerates for surfactants (Figure 2.7(B)). Therefore, the smaller and larger values of Dh were indicated to the presence of spherical micelles and micellar agglomerates, respectively, which are in good agreement with previously reported studies.[34,41,43]

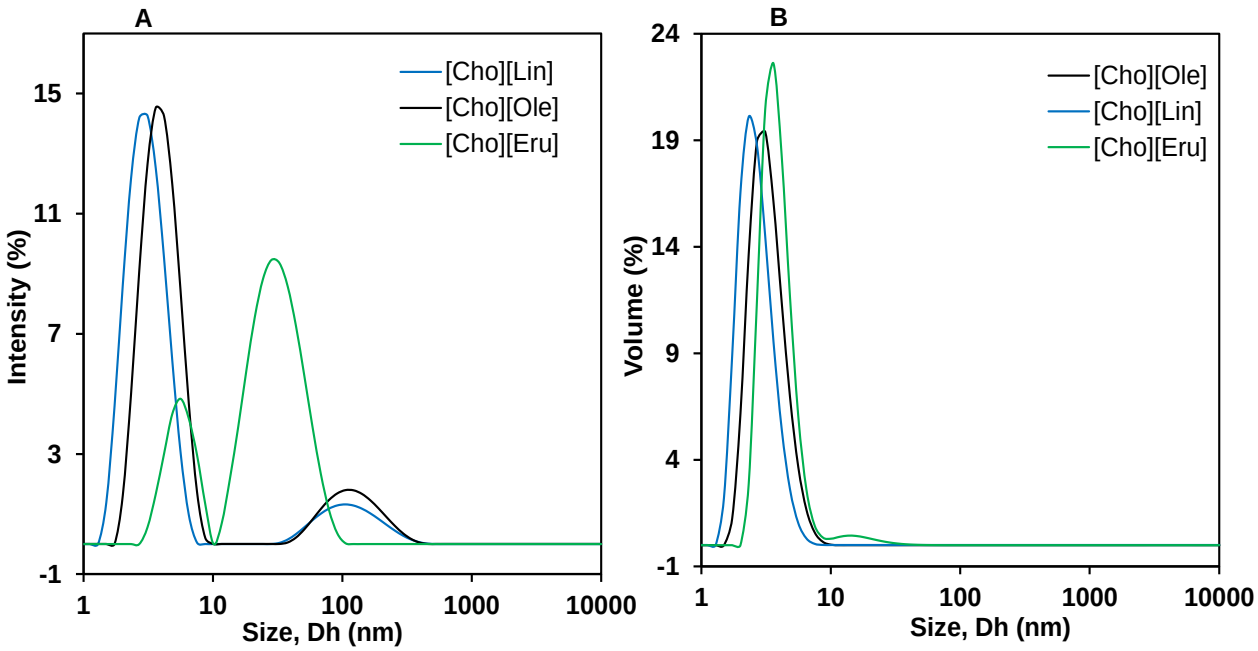


Figure 2.7. Micelle sizes and size distributions of the choline-based IL surfactants in term of (A) %intensity and (B) % volume.

2.4.5. Cytotoxicity of the synthesized SAILs

To evaluate the biocompatibility of the synthesized SAILs, mammalian cell line NIH 3T3 was used to investigate their cytotoxicities and the results were then compared with those of the conventional surfactants Tween 80 and SDS. A mathematical logarithm function was used to calculate the IC_{50} values (Figure 2.8). The synthesized SAILs showed lower toxicity than the traditional ionic surfactant SDS. The IC_{50} values of [Cho][Ole], [Cho][Lin], [Cho][Eru], SDS,

and Tween 80 are 0.59, 0.33, 0.29, 0.16, and 0.68 mM, respectively. The toxicity mainly depends on the alkyl chain lengths of the cation and anion. It has been reported that there is no intrinsic toxic effect of cations such as sodium, potassium, and choline on HeLa or SK-Mel-28, at least in the relevant range of concentrations.[19] Therefore, the toxicity of the investigated surfactants is mostly derived from the anions.

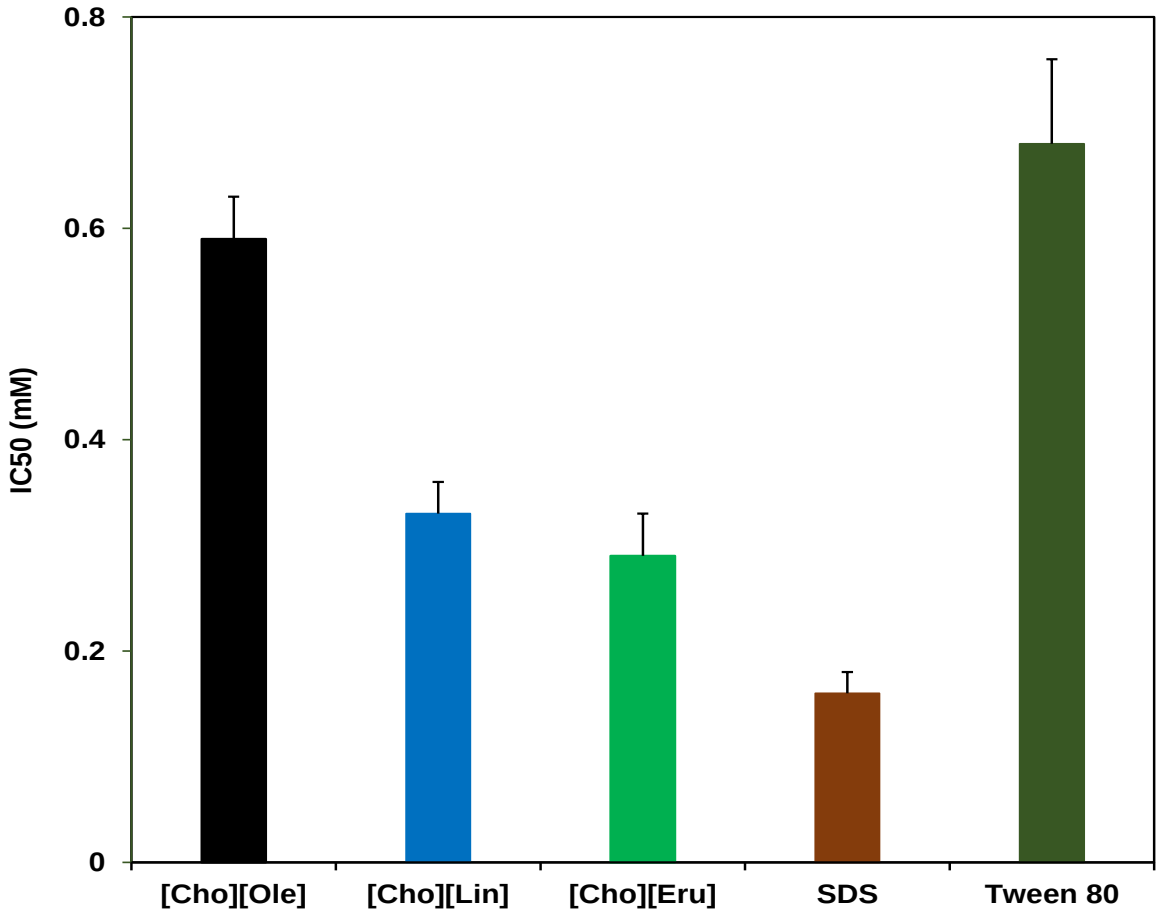


Figure 2.8. Cytotoxicities of the choline–fatty acid ILs and traditional surfactants.

The [Cho][Eru] surfactant has higher toxicity (0.29 mM) than the other SAILs and Tween 80, but it is lower than that of the traditional surfactant SDS (0.16 mM). The long alkyl chain of the erucate anion causes penetration enhancement of anions into the cell membrane, leading to rapid disturbance of the cell structure.[23,24] [Cho][Lin] has higher toxicity than [Cho][Ole], even though the number of carbon atoms is the same. The presence of an extra double bond in [Cho][Lin] increases the toxicity because of the strong π–π interaction between the Lin anion and the cell membrane, which causes more cell death. [Cho][Ole] has the lowest toxicity among the SAILs, and it is almost similar to that of the conventional surfactant Tween 80. This result is in good agreement with previously reported study (IC_{50} of [Cho][Ole] in HeLa and SK-Mel-

28 are 0.47 mM and 0.52 mM, respectively).[21] Therefore, the SAILs are potential low-toxicity alternatives to traditional ionic surfactants.

2.5. Conclusions

In this study, a series of potentially low toxic and halogen free choline–fatty-acid-based surface-active ionic liquids (SAILs) were synthesized and their aggregation behavior in aqueous medium was evaluated. The CMC and γ_{CMC} values of the SAILs in water were found to be lower notably than those of the conventional ionic surfactants, SDS and SDBS due to the presence of longer hydrophobic alkyl chain or/and size difference of the cations (Cho^+ and Na^+), which are less hydrated by water and provide the strong electrostatic repulsion between the charged head groups in the surface layer.[6,7,33,38] The thermodynamic properties of micellization (ΔG^0_m, ΔH^0_m, and ΔS^0_m) indicated that the micellization process of the SAILs is spontaneous, exothermic, and entropy-driven at room temperature. The dynamic light scattering measurement indicated the formation of spherical micelles with a range of 3.9-5.6 nm in diameter. Furthermore, the toxicity profile of [Cho][Ole] falls in the range of practically harmless (similar toxicity to Tween 80). [Cho][Eru] is more toxic than [Cho][Ole], which is less toxic than some traditional surfactants.[21] We believe that this work would open up new possibilities for the development of IL-based biocompatible surfactants for various fields, including biological and biomedical applications.

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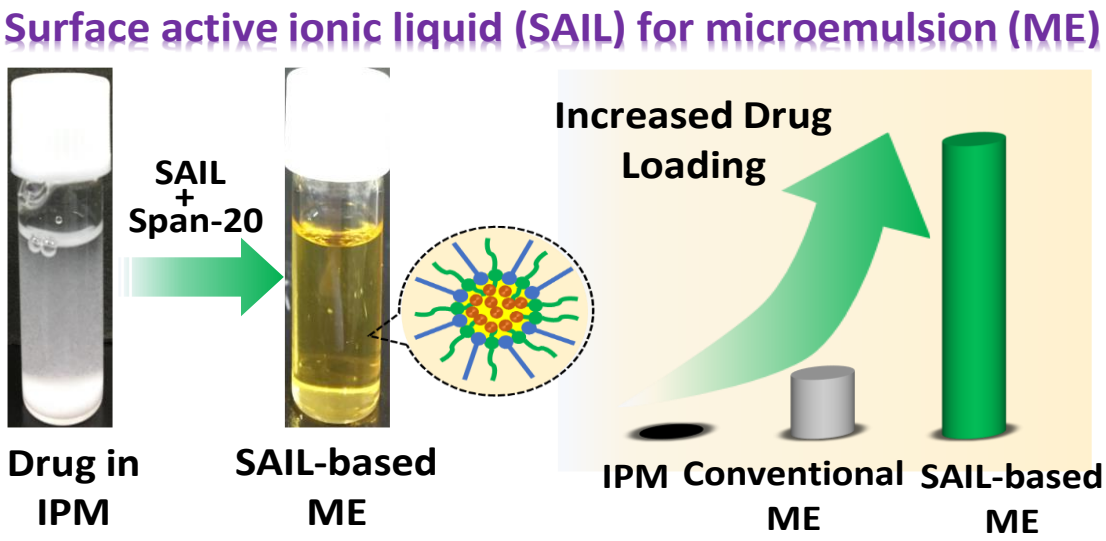
CHAPTER 3: BIOCOMPATIBLE IONIC LIQUID SURFACTANT BASED MICROEMULSION AS A POTENTIAL CARRIER FOR SPARINGLY SOLUBLE DRUGS



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3.1. Abstract

Developing a universal drug delivery vehicle of sparingly soluble drugs remains a challenge, with surface-active ionic liquid (SAIL)-based ionic liquid-in-oil (IL/O) microemulsions (MEs) being the most suitable vehicles. In this study, a series of SAILs were formulated to prepare novel IL/O MEs composed of SAIL, sorbitan laurate (Span-20) and isopropyl myristate. Based on the constructed pseudo-ternary diagrams, the SAILs played vital surfactant roles with Span-20 acting as a co-surfactant. Excellent drug solubility of MEs prepared with a ratio of 2:1 (SAIL[Cho][Ole]: Span-20) was observed. Examination of the droplet shape, size and size distribution of the MEs revealed well-distributed particle sizes of 6.5 to 21.2 nm that formed spherical micelles with the IL 1,3-dimethylimidazolium dimethyl phosphate at the core of the MEs. The MEs showed excellent solubility of sparingly soluble drugs (i.e., celecoxib, acyclovir, methotrexate and dantrolene sodium). In vitro cytotoxicity of the new carrier using a three-dimensional reconstructed human epidermis model revealed that cell viability of SAIL-based MEs (94%) was similar when compared with conventional Tween-80 based MEs (96%) at the same IL concentration (4%). The results indicate that the SAIL surfactant in the MEs represents a potential alternative to conventional surfactants for solubilizing insoluble drug molecules.

3.2. Introduction

Microemulsions (MEs) have attracted significant interest as promising smart drug delivery carriers for topical and transdermal drug delivery (TDD) because of their straightforward preparation, long-term stability, biocompatibility and high drug solubilization capacity for potential biomedical applications.[1–3] In general, MEs are a class of transparent, isotropic, thermodynamically stable micro heterogeneous mixtures consisting of two immiscible liquids (polar and nonpolar) that are stabilized by an interfacial film of surfactant with the combination of a cosurfactant.[4–6] MEs are capable of solubilizing a wide range of polar and nonpolar substances in their nanodomains and are used widely in the fields of drug delivery systems, chemical reactions, extractions and nanomaterial preparations.[7,8] Interestingly, ME-based drug delivery is a potential approach for formulating sparingly soluble drugs that have limited solubility and permeability and are difficult to formulate with conventional delivery systems.[9] Both the water-in-oil (W/O) and oil-in-water (O/W) type of MEs are now used extensively for developing effective delivery systems of these drugs, which are neither soluble in water nor in oil.[1,10] Conventionally, these MEs have been formulated using water as the polar core and then the polar component is combined with standard anionic surfactants such as sodium 1,4-bis (2-ethylhexyl) sulfosuccinate;[4,11,12] however, these MEs have inadequate thermal stability. Non-

aqueous MEs have been tested to improve thermal stability, where the aqueous polar core is replaced by several non-aqueous polar components such as acetonitrile, methanol, ethylene glycol, glycerol and formamide.[10,13] However, these solvents are not acceptable pharmaceutical ingredients because of their toxicity, skin irritation and non-biocompatibility properties.[14,15]

In the last decade, ionic liquid (ILs)-based MEs have been tested as potential nanocarriers for TDD. ILs are tunable designer solvents and play a versatile role at all phases of ME systems by altering water, oil and surfactant components.[16–20] Moniruzzaman et al. developed IL-in-oil (IL/O) MEs where IL replaced the water phase for solubilizing sparingly soluble drugs acyclovir (ACV) and methotrexate (MTX).[19] Subsequently, they evaluated the dermal delivery of 5-flurouracil through an IL/O system and investigated the influence of the surfactants ratio in an effort to promote their application in pharmaceutics.[21] Recently, researchers have introduced ILs as a surface-active IL (SAIL) to serve as a surfactant by combining cations and anions with long alkyl chains for formulating different organized assemblies and improving the physico-thermal stabilities of MEs over aqueous conventional systems.[22,23] It has been reported that the SAILs are possessed better surface-active properties, enhanced antimicrobial and skin permeation activity, high temperature stability and temperature insensitivity, and even they considered as environmentally friendly surfactants (amino acids and choline containing SAIL) as compared to conventional surfactants.[8,24–26] When the non-ionic conventional surfactants such as tween-80 are used to prepare MEs, they are not stable at high temperature as compared with ionic surfactants.[8,27] However, most of the SAILs are ionic and able to solubilize hydrophilic ILs, which facilitates the generation of stable IL/O ME formulations over a wide temperature range.[28,29] The SAILs also possess combined properties of ILs and surfactants. Recently, Zech et al. formulated and characterized a SAIL-containing ME (1-hexadecyl-3-methylimidazolium chloride) where 1-butyl-3-methylimidazolium tetrafluoroborate and ethylammonium nitrate were used as polar components and dodecane as the non-polar component,[20] and Rojas et al. formulated and characterized a SAIL-containing ME (1-butyl-3-methylimidazolium dodecylsulfate) composed of 1-ethyl-3-methylimidazolium ethyl sulfate as the polar medium and toluene as the non-polar medium.[30] However, the use of volatile organic solvents as the non-polar phase in SAIL-containing MEs is unsuitable because of their high toxicity and non-biodegradable nature.[14] Safavi et al. have investigated 3-octyl-1-methylimidazolium chloride as a SAIL-containing ME where a hydrophobic IL, 1-butyl-3-methylimidazolium phosphate, was used as a substitute for the organic solvent and water as the polar component.[31] However, this SAIL and other SAILs are not suitable for potential applications because toxic cations such as ammonium, imidazolium, pyrrolidinium and

morpholinium are used.[32–34] To address these issues, eco-friendly and biodegradable cholinium fatty acid or amino acids containing SAIL-based MEs are required, where the SAILs are potential alternatives to conventional surfactants for envisaging biomedical applications.[35,36]

The aim of this study was to develop a cholinium fatty acid IL-assisted non-aqueous ME by replacing Tween-80 and thus introduce a promising alternative to conventional surfactants. In our earlier report, three cholinium fatty acid IL surfactants were synthesized as potential green alternatives to traditional ionic surfactants, and their micellar behavior and toxicity were investigated.[32] In the present study, biocompatible ionic liquid surfactant based IL/O MEs were prepared and stabilized by a blend of IL, which served as a solubilizing agent, and a mixture of two surfactants, Span-20 and cholinium fatty acid IL, in isopropyl myristate (IPM). To build the ideal IL/O ME composition, a pseudo-ternary phase diagram was prepared by varying the weight ratio of surfactant mixtures and the IL component. The prepared formulation was characterized by using dynamic light scattering (DLS) to optimize the desired weight ratio of surfactant mixtures and IL component in IPM. *In vitro* cytotoxicity of the selected ME formulations was examined using a three-dimensional reconstructed human epidermis model. The encapsulation efficiency of drugs into the ME carrier was also determined. Finally, the stability of drug loaded MEs was compared with conventional Tween-80-based W/O MEs to examine whether the SAIL surfactant represents a promising alternative to traditional surfactants.

3.3. Materials and methods

3.3.1. Materials

Wako Pure Chemical Industries (Osaka, Japan) supplied oleic acid, linoleic acid and erucic acid with high purity. Commercially available Tween-80 (polyoxyethylene sorbitan monooleate), IL[C_1mim][$(MeO)_2PO_2$] (1,3-dimethylimidazolium dimethyl phosphate) and Span-20 (sorbitan laurate) were purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification. ASPION pharmaceuticals (Kobe, Japan) supplied ACV as a generous gift. MTX and 1-[(5-(p-nitrophenyl) furfurylidene) amino] hydantoin sodium (dantrolene sodium) were also obtained from Wako Pure Chemical Industries. Celecoxib and isopropyl myristate (IPM) were obtained from Tokyo Chemical Industries (Tokyo, Japan). All other analytical grade reagents were used in the experiments without further purification. The reconstructed human epidermal model, LabCyteTM EPI-MODEL 24, was purchased from

Japan Tissue Engineering (Aichi, Japan). MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) medium was purchased from Dojindo (Kumamoto, Japan).

3.3.2. Screening of the ME Formulation Ingredients

In this work, various ingredients were selected to develop a novel ME. A well-known continuous phase, e.g., IPM, that is pharmaceutically accepted and used extensively for ME formulations was selected.[14] To study the effect of the ionic property of surfactants on miscibility in IPM three SAILs, Cholinium Oleate ([Cho][Ole]), Cholinium Linoleate ([Cho][Lin]) and Cholinium Erucate ([Cho][Eru]), were synthesized and screened. Initially, SAIL and Span-20 were blended at a fixed weight ratio. The required amount of IPM was then added to produce a surfactant concentration of 20 wt.% in IPM, and the mixture was then stirred to gain a macroscopically homogeneous solution. The maximum solubility of IL[C_1mim][$(MeO)_2PO_2$] was then determined by titration until the solution was turbid. Moreover, the compatibility of the components was visually examined.

3.3.3. Phase Behavior Study

The mixtures of SAILs and Span-20 were taken in ratios (in weight fraction) of 2:1, 1:1, 2:3 and 1:3 to study the phase behavior. The mixtures were then dissolved in IPM to produce surfactants/IPM systems. The total surfactant concentration was varied from 5 to 70 (wt.%). The maximum encapsulation of IL was determined by adding IL continuously until the solution was turbid.

3.3.4. Encapsulation of IL in SAILs/Span-20/IPM System

For encapsulation studies of IL, the surfactants were selected from phase behavior study and mixed in several combinations to obtain defined surfactant mixtures at fixed weight ratios of 2:1, 1:1, 2:3 and 1:3. To prepare a macroscopically homogeneous solution, 10 and 20 wt% of each mixture was added in the required amount of IPM and the mixtures were stirred. The maximum encapsulation of IL[C_1mim][$(MeO)_2PO_2$] was determined by titration until the clear oil ME solution changed to a turbid solution at room temperature (25 °C). The enhancement of IL uptake into the system was achieved by stirring and settling at each addition (w/w) of IL.[14,37]

3.3.5. Preparation of MEs with an IL

To characterize an IL in oil (IL/O) ME, the surfactant mixtures were selected based on the solubility studies. The two selected surfactants were SAIL[Cho][Ole] and Span-20. The blend of SAIL[Cho][Ole] and Span-20 was prepared at a fixed weight ratio of 2:1. Here, we prepared

two types MEs containing 10 or 20 wt% of the surfactant mixtures. The surfactant mixtures were then added to IPM and an optically transparent solution was prepared by mixing thoroughly. The IL was added to the solution and mixed vigorously to prepare the IL/O ME.

3.3.6. Measurement of the ME Droplets Size

The mean sizes and size distributions of ME droplets were examined by DLS using a Malvern Zetasizer Nano ZS light scattering instrument (Malvern Instruments Ltd., Malvern, Worcester, UK) with a 633 nm laser. Samples were filtered through a 0.45 μm Millipore Millex-LG filter to remove any traces of dust or contaminants. Data were collected after 10 minutes equilibration. The average of five replicated measurements provided the mean diameter of ME droplets. The Malvern DTS software was used to measure the droplet size and polydispersity of the dispersed IL phase.

3.3.7. Stability of MEs

The physical stability of the new SAIL-contained non-aqueous MEs was monitored over different periods and temperatures by measuring droplet diameter variations and visual inspection at regular intervals. Initially, we prepared MEs containing SAIL[Cho][Ole]/Span-20/IPM/IL at several IL contents and these MEs were stored at 25 °C. The stability of the prepared MEs were determined by measuring droplet sizes of the MEs by DLS. The physical stability of MEs was examined by centrifugation at 10,000 *g* for 15 min at 25 °C. Additionally, the extent of degradation and encapsulation efficiency of the drugs was examined to determine the chemical stability of IL/O ME formulations.

3.3.8. In vitro Cytotoxicity Studies

The reconstructed human epidermal model LabCyte EPI-MODEL 24 was used to determine the cytotoxicity of MEs, as described previously with some modifications.[9,38] Initially, tissues were placed into 24-well plates (BD Biosciences, San Jose, CA, USA) through assay medium (0.5 mL) and then incubated overnight at 37 °C in a 5% CO_2 atmosphere. Twenty-five microliters of each test sample was added to the tissue surface and the cultures were then incubated for 24 h (37 °C, 5% CO_2, humidified environment). At the end of the 24 h incubation period, the epidermal tissues were carefully washed 15 times with D-PBS to remove any remaining test samples from the tissue surface. Freshly prepared (0.5 mL) MTT medium (0.5 mg mL^{-1}) was then added to the wells and the tissues were incubated for 3 h at 37 °C, 5% CO_2, humidified atmosphere. Each epidermal tissue fragment was then transferred into 0.3 mL 2-propanol (microtubes were used) and the epidermal tissue was fully immersed in this solution.

The microtubes were placed in the dark at 4 °C for 2 d to extract the dye. Subsequently, the extracted solution from the microtubes was transferred into 96-well plates and the absorbance was measured at 570 and 650 nm using a 96-well plate reader. Two-propanol was used in one well as a blank and IPM was used as a negative control. Percentage cell viability was calculated against the relative viability of the negative control.

3.3.9. Dissolution of Drugs in MEs

Drug solubility in MEs was determined by the following procedure.[14,39] Initially, we selected four model drugs: celecoxib (CLX), ACV, MTX and dantrolene sodium (DTS). Excess amounts of the drugs were added to water, IPM and IL/O MEs. All samples were kept for 48 h at 25 °C with continuous stirring. A 0.45 μm Millipore Millex-LG filter was then used to remove the precipitated drug by filtration. The solubility of the drugs was determined by measuring the absorbance at 252, 254, 302 and 380 nm for CTX, ACV, MTX and DTS, respectively. All samples were diluted with methanol, which was used as the blank.

3.4. Results and discussion

3.4.1. Selection of the ME Formulation Ingredients

The components of pharmaceutical MEs play a vital role in forming a stable vehicle, enhancing drug solubility, and ensuring a high level of drug permeation through skin. Moreover, the selected components of MEs should be non-toxic and biocompatible for developing a smart transdermal drug delivery. In this study, a series of biocompatible IL surfactants were selected as potential alternatives to the conventional surfactant, Tween-80, and blended with a non-ionic surfactant, Span-20 (as an emulsifying agent and as cosurfactants[40]) to become miscible with oil phases. Generally, the most commonly used ingredients, such as IPM and Span-20, are pre-selected as the pharmaceutical accepted ingredients because of their stability and non-toxicity in ME formulations.[9,14] Cholinium fatty acid-based SAILs have been reported to show superior surface activity and lower toxicity when compared with conventional surfactants.[35,41–44] As a promising substitute of the conventional surfactant (Tween-80), the micellar behavior and toxicity of cholinium fatty acid IL surfactants, such as SAIL[Cho][Ole], SAIL[Cho][Lin] and SAIL[Cho][Eru], have been reported previously.[32] SAIL[Cho][Ole] showed lower toxicity when compared with that of conventional surfactants and analogous ILs. In addition, most of the SAILs are ionic and able to solubilize hydrophilic ILs, which facilitates the generation of stable IL/O ME formulations.[20,30,31] The hydroxyl groups of Span-20 or choline cations in IL

surfactants form multiple hydrogen bonds with the head groups of surfactants.[14,37] In contrast, a series of the hydrophilic IL have been reported as a disperse phase in IL/O MEs formulation, which improves drug solubility and the formation of stable ME droplets.[14] Here, the hydrophilic IL [C_1mim][$(MeO)_2PO_2$] was used as the non-aqueous phase to ensure low toxicity and facilitate greater solubility efficacy and H-bonding ability for stable ME formation.[9,37,45] IPM was used widely as the oil phase or vehicle for drug formulation and shows good compatibility with Span-20 and Tween-80. In this study, IL/O MEs were formulated using various weight ratios of SAILs and Span-20 as a potential alternative to conventional Tween-80. The phase behavior study of SAILs showed the prepared MEs could encapsulate a significant amount of IL.

3.4.2. IL Encapsulation in SAILs/Span-20/IPM Systems

To check the encapsulation of the IL[C_1mim][$(MeO)_2PO_2$] in SAILs-based MEs, the IL/O MEs were formulated by dispersing 10 or 20 wt% of SAILs + Span-20 in IPM. Generally, the encapsulation of ILs increases with increasing concentration of surfactant mixtures because the hydroxyl groups of surfactants have strong electrostatic interactions with cationic ILs. Additionally, there are multiple hydrogen bonding sites on the head groups of surfactants that enhanced the encapsulation of ILs into MEs cores.[14] The maximum encapsulation of IL in each new ME is shown in Figure 3.1. When the concentration of SAILs in MEs was gradually increased with a fixed total surfactant concentration (20%), IL encapsulation also increased because of the increase in the interfacial region of total surfactants (SAIL + Span-20) with increasing SAIL concentration.[46] Hence, encapsulation of this IL was enhanced at the higher total surfactant mixtures (20%) when compared with that of the lower total surfactant mixtures (10%). Encapsulation of IL in SAIL[Cho][Ole] based MEs (98.8 mg/g) was two times higher than that of Tween-80 based MEs (47.1 mg/g) at a fixed surfactant mixture (i.e., 20%).These results suggest the presence of strong electrostatic and hydrogen bonding interactions between SAILs and IL [C_1mim][$(MeO)_2PO_2$].[9] Among the SAILs used, the SAIL[Cho][Ole] surfactant gave higher IL encapsulating capacity than SAIL[Cho][Lin] and SAIL[Cho][Eru], which contain two double bonds and a long alkyl chain (C_{22}) in their structures, respectively. H-bonding interactions have been reported to decrease with increasing numbers of van der Waals interactions between alkyl chains of SAIL surfactants.[47–49] However, the superior encapsulation of IL in the SAIL[Cho][Ole] surfactant can be attributed to dual interactions, i.e., electrostatic interactions between the hydroxyl groups and imidazolium cation and hydrogen bonding between the hydroxyl groups of this IL with the head groups of the surfactants.[14]

Therefore, the IL may acts as a ‘‘glue” to bond with surfactants head groups and play vital role as the driving force for the formation of stable ME droplets in IPM with a mixture of SAILs and Span-20.[14]

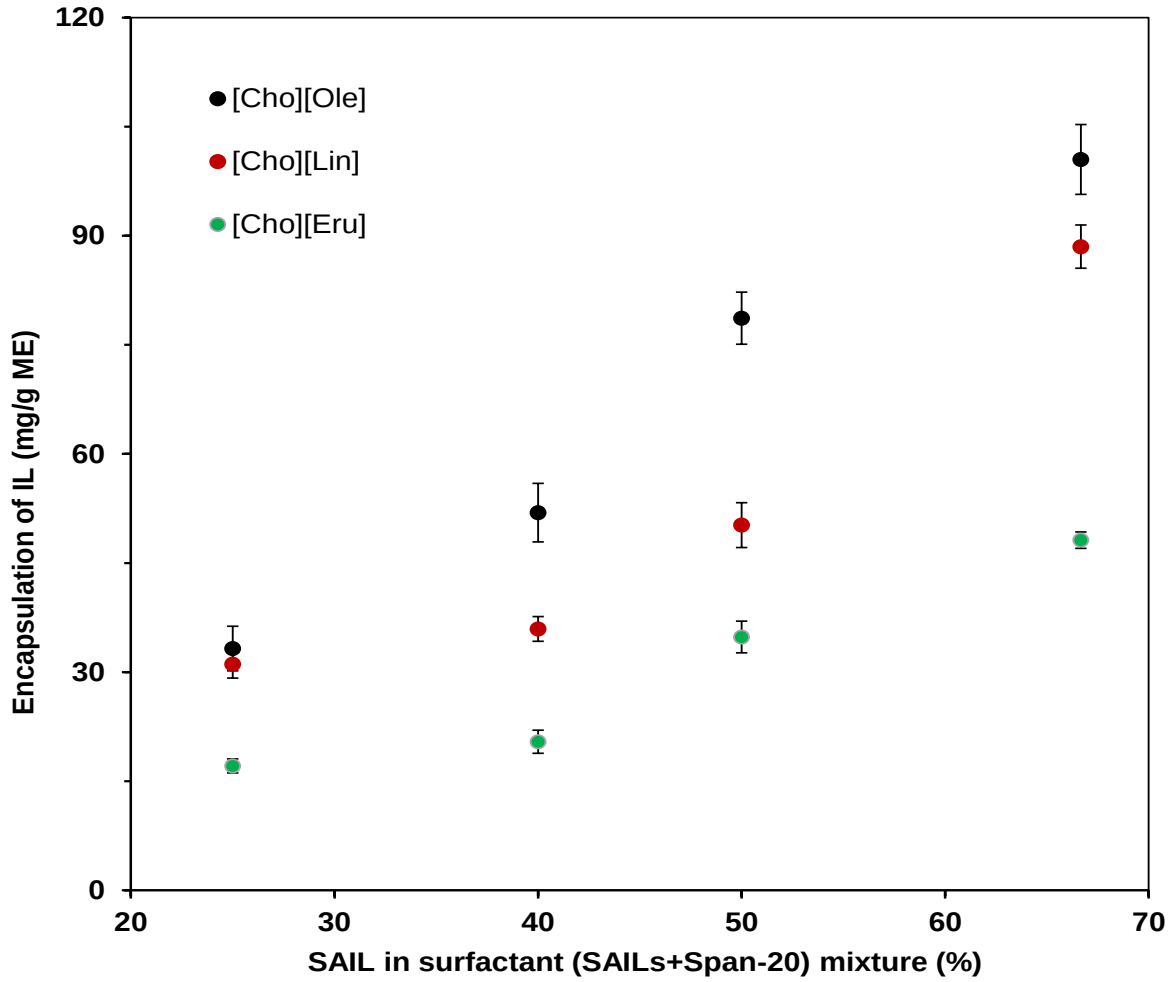

Figure 3.1. Encapsulation of IL in MEs with increasing SAIL concentration at a 20 wt% total surfactant concentration (SAIL + Span-20).

3.4.3. Phase Behavior

Pseudoternary phase diagrams of IL encapsulation were generated using different weight ratios of ME forming components to further evaluate the phase behavior of IL/O MEs. As expected, the encapsulation of IL in one surfactant system (SAIL or Span-20) was limited even at higher surfactant concentrations when compared with that of a blend of two surfactants (data not shown) that have good agreement with conventional ME systems. The second surfactant may reduce the interfacial tension among the ME components to increase interface fluidity, which aids formation of flexible MEs instead of rigid liquid crystalline structures.[14] Four ME formulations with different weight ratios of ILs, SAILs + Span-20 and IPM were examined to

calculate the ME region for forming a stable ME system. Figure 3.2 represents the phase diagram of the SAIL[Cho][Ole] surfactant based MEs prepared with weight ratios (w/w) of 2:1, 1:1, 2:3 and 1:3 at 25 °C. The ME region increased significantly throughout the diagrams as the SAIL[Cho][Ole] surfactant concentration increased. The area of the single-phase zone varies with the SAIL[Cho][Ole]/Span-20 ratio in the order 2:1 > 1:1 > 2:3 > 1:3, which indicated that formation of MEs with IL as a polar core was assisted by gradual increase in the concentration of SAIL[Cho][Ole] surfactant.

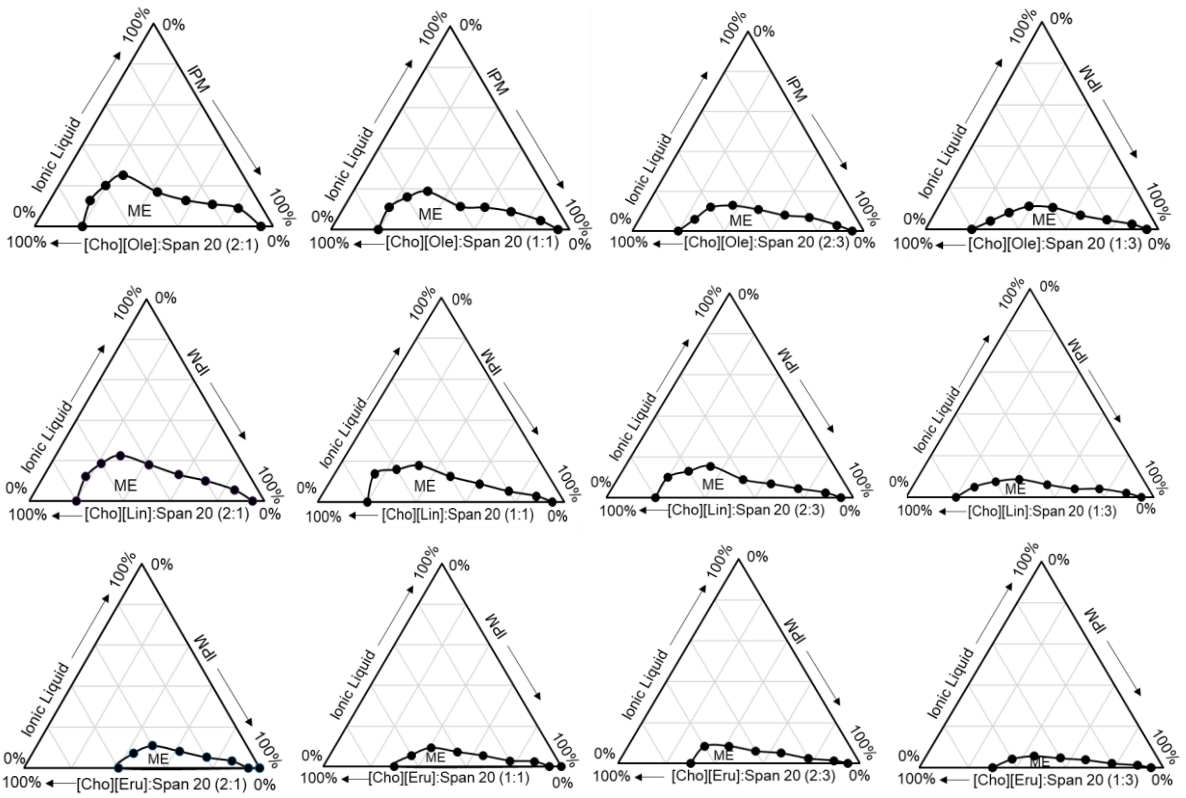

Figure 3.2. Pseudoternary phase diagram of IL[C_1mim][$(CH_3O)_2PO_2$]/(SAILs+ Span-20)/IPM ME systems at 25 °C.

A similar trend was observed when phase diagrams were built with weight ratios (w/w) of 2:1, 1:1, 2:3 and 1:3 at 25 °C for SAIL[Cho][Lin] and SAIL[Cho][Eru] surfactants (Figures 3.2 and). Interestingly, when the ratio SAIL:Span-20 was fixed at 2:1, the SAIL[Cho][Lin] and SAIL[Cho][Eru] surfactants exhibited a smaller ME forming area than the SAIL[Cho][Ole] surfactant (Figures 3.2) because of the presence of two double bonds and long alkyl chain (C_{22}) in their structures, respectively that decrease the intermolecular interaction i.e. H-bonding contact with increasing the number of van der walls interactions between long alkyl chains of surfactants.[47,49] Additionally, a lower weight fraction of SAIL based surfactant mixtures was required to solubilize a large amount of IL into ME cores (75 mg/g IL soluble in 10% total

surfactant at a weight ratio 2:1), which is comparable with previous studies using Tween-80 (0.0 mg/g in same specific formulation).[14,37] These interesting findings can be elucidated in terms of the promising interfacial properties (e.g., rigidity, polarity) delivered by the mixture of two different surfactants.

3.4.4. ME Droplet Measurements

The shape, sizes and size distribution of the ME droplets were measured at 25 °C by using DLS. The DLS measurement also assesses the ILs encapsulation capability of the SAIL surfactant to create MEs. The formulation of ME droplets at different IL contents was verified by the IPM/surfactant mixture (SAIL [Cho][Ole] + Span-20)/IL([C_1mim][$(CH_3O)_2PO_2$]) ternary systems. The total surfactant concentration of the ME system was kept constant at 20 wt.%. Figure 3.3(A) shows the variation in the size and size distribution of IL droplets with increasing *R* value, where *R* is the molar ratio of ILs to SAILs. As expected, the micelle volume of MEs increased with increasing IL content in MEs, which is in agreement with results for conventional IL/O MEs.[3,14] Micelle diameters of 6.5 to 21.2 nm were obtained, and are in good agreement with typical reverse micelles with water cores that are stabilized by common surfactants.[50–52]

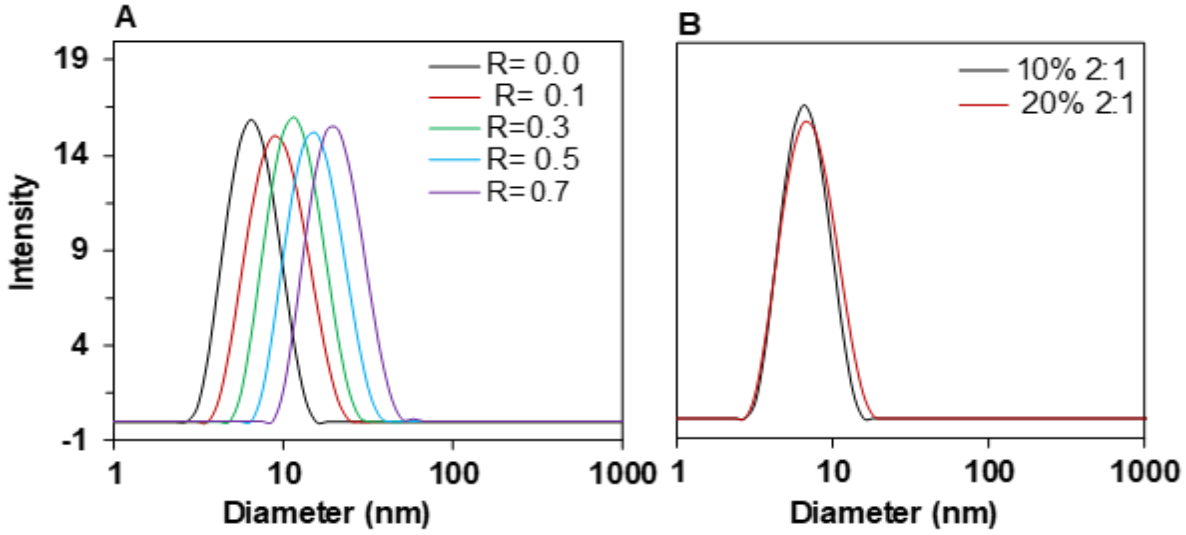

Figure 3.3. DLS results showing the sizes and size distributions of IL droplets in the SAIL [Cho][Ole] based ME system (A) with different *R* values and (B) with different total surfactant concentrations (*R* = 0.2) at 25 °C.

The observed sizes of IL/O MEs are significantly lower than that of [C_2mim][BF_4]/TX-100/oil MEs, where droplet sizes are on the order of 0.1 μm.[12,52] However, increase in the size of the droplets from 6.5 to 21.2 nm (linearly with increasing *R* values) demonstrated clearly the formation of IL/O MEs through IL encapsulation. When the total concentration of the surfactant was increased at a constant *R* value, the mean micelle size of MEs did not change significantly, attributing a constant size of micelle formation (Figure 3.3(B)). This results also indicates the existence of discrete and non-interacting spherical IL droplets in ME media that are stabilized by the SAIL surfactant.[14] The shape of the MEs with IL domains was also investigated by DLS. The size of the ME droplets varies with increasing IL content in ME media. According to the swelling law of MEs, the droplets would be spherical if the size of the droplets follows a linear relationship to IL content.[51,53] The hydrodynamic diameter of IL droplets was found to vary almost linearly with increasing IL content, indicating a spherical micelle structure (Figure 3.4).

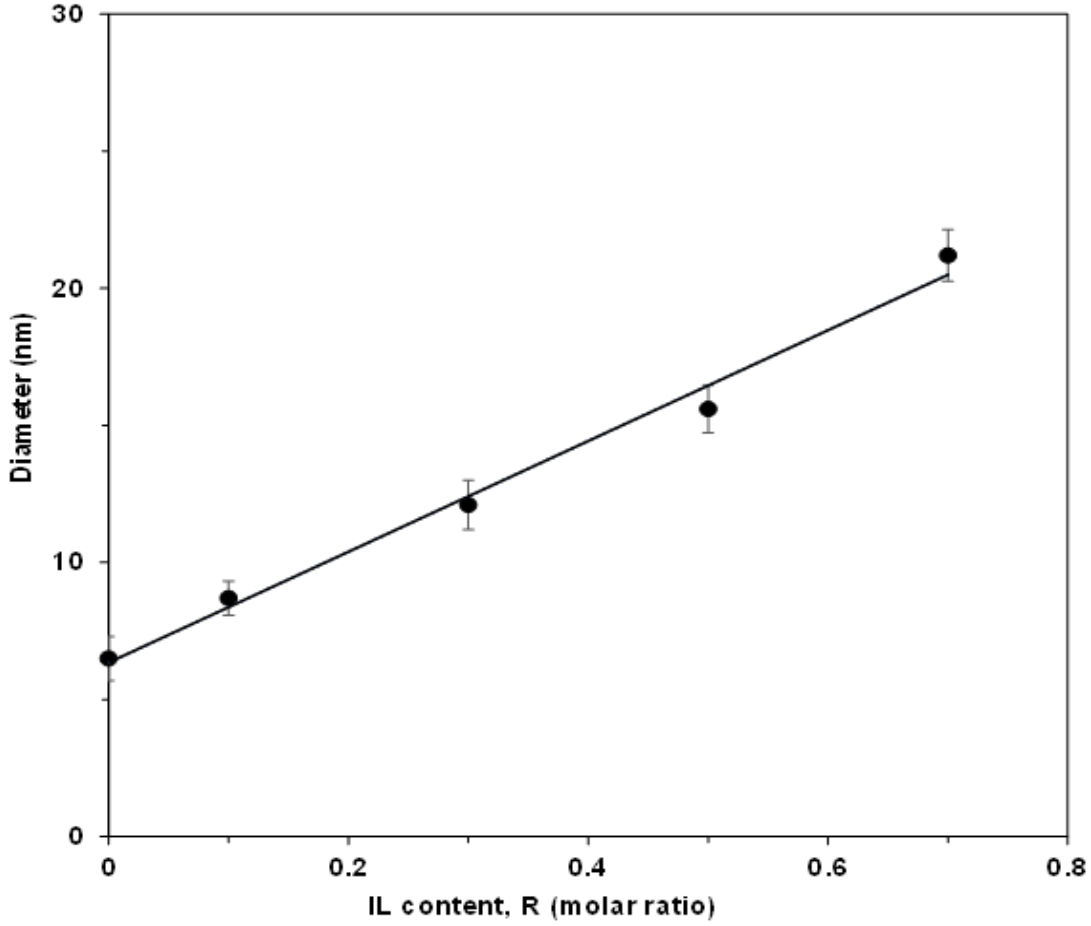

Figure 3.4. The diameter of the MEs droplets (measured by DLS) at an IL to surfactants (SAIL [Cho][Ole] + Span-20) molar ratio at 0.2.

3.4.5. Physical Stability of MEs

The physical stability of the nanocarrier is very important in the design of ME-based delivery systems for food, cosmetic and pharmaceutical products. We therefore examined the stability of the ME formulation for an extended period by visually inspecting for any phase separation and monitored droplet diameter changes over the storage period. For this analysis, the surfactant (SAIL[Cho][Ole] + Span-20)/IPM/IL ([C_1mim][$(CH_3O)_2PO_2$]) MEs at various IL contents were prepared and stored at room temperature (approx. 25 °C). The IL/O MEs were stable up to 120 d because no turbidity, phase separation or precipitation was observed. As shown in Figure 3.5(A), the droplet sizes and size distributions of IL/O MEs showed no significant change after 45 d, indicating that the proposed IL/O ME system is physically stable.[8,14]

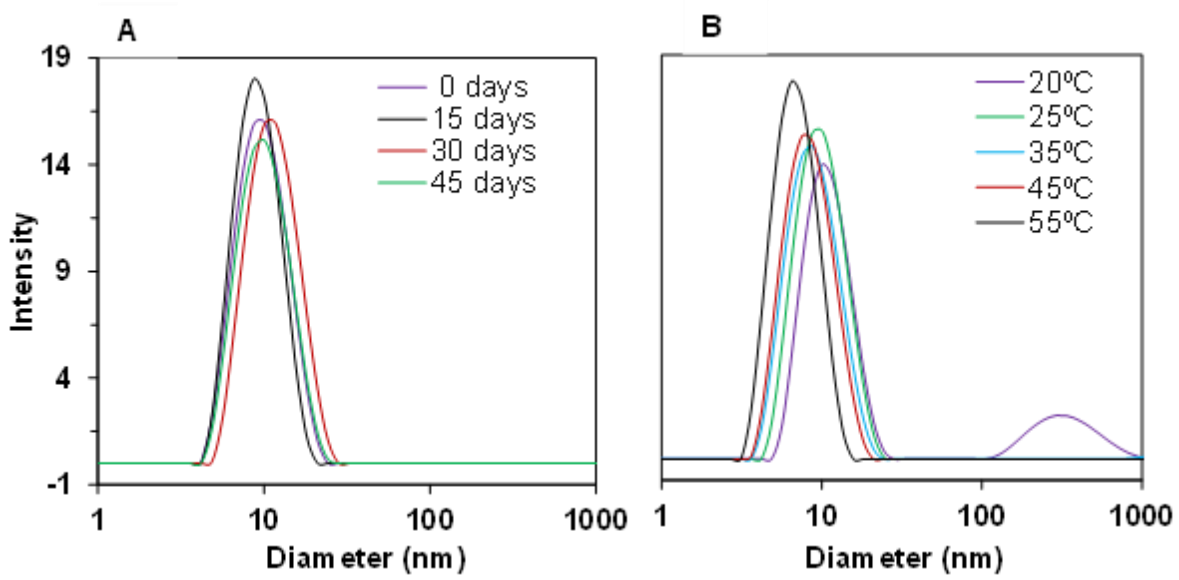

Figure 3.5. The hydrodynamic diameter of the SAIL [Cho][Ole] based MEs with R = 0.3 was evaluated as a function of time (A) and temperature (B) to evaluate physical stability.

We also evaluated the effect of temperature on the size of droplets in surfactant (SAIL [Cho][Ole] + Span-20)/IPM/IL ([C_1mim][$(CH_3O)_2PO_2$]) MEs at R = 0.3 by DLS (Figure 3.5(B)). Generally, the size of the droplets in MEs decreased with increasing temperature, and vice versa at specific surfactant ratios, indicating that the ME droplets behave as non-interacting hard spheres.[4,8,53] The size of the prepared MEs decreased with increasing temperature from 20 to 55 °C, indicating the absence of any droplet-coalescing properties among the IL/O MEs.[4,51,54] Interestingly, the prepared formulations at R = 0.3 were stable at temperatures as low as 20 °C where no phase separation was observed from 20 to ~55 °C, suggesting the MEs are stable at high temperatures. In addition, the size of ME droplets at R =

0.3 reduces from 10.1 to 6.3 nm when the temperature of the sample is increased. This result suggests that the MEs are capable of retaining their structural integrity up to ~55 °C.[8] These new SAIL based MEs are promising carriers of drugs, cosmetics and food formulations, especially when water sensitive compounds are present.

3.4.6. In vitro Cytotoxicity Studies

In vitro cytotoxicities of SAIL and Tween-80 surfactant-based MEs were carried out to evaluate the relative safety effect of the MEs formulations using EPI-MODEL 24. Using IPM as the control, we compared cell viability among IL (4% IL/96% IPM), the SAIL-based ME1 (20% (SAIL[Cho][Ole]:Span-20 (2:1))/4% IL/76% IPM), conventional Tween-80 based ME2 (20% (Tween-80:Span-20 (2:1))/4% IL/76% IPM) and ME3 (20% (Tween 80:Span-20 (2:1))/4% water/76% IPM) formulations. Generally, most common ILs with short alkyl chains that are affixed to the amino acid or imidazolium cation exhibit low toxicity and better biodegradability.[9,55,56] The usage of IL [C_1mim][$(CH_3O)_2PO_2$] in MEs is favorable for developing drug delivery systems because of their lower toxicity,[14] which is also reflected in our current study.

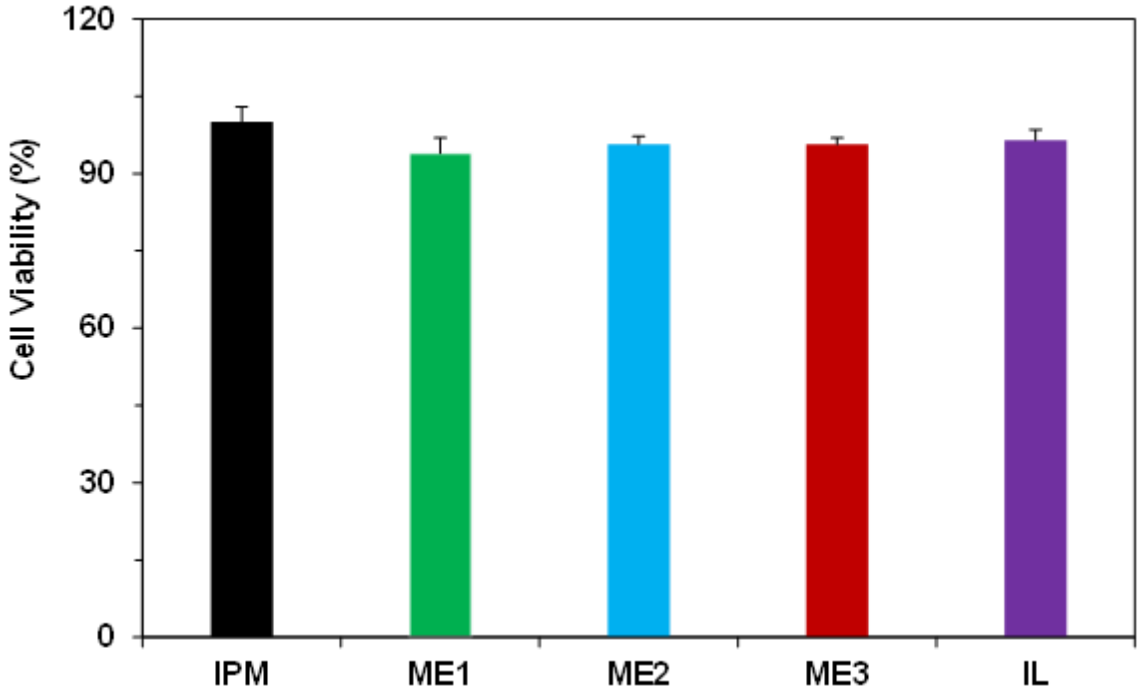

Figure 3.6. *In vitro* cytotoxicities of different formulations according to the MTT assay (*n* = 3).

The cell viability when using 4% IL was 97% when compared with that of the control experiment (100% IPM). Such low toxicity was also observed when conventional Tween-80

based W/O MEs, i.e., 4% IL or 4% water containing W/O MEs (ME2 or ME3 in Figure 3.6), were used. The cell viability of the SAIL-based ME formulations (both ME1 and ME2) showed almost similar toxicity when compared with that of Tween-80-based MEs at the same IL concentration (4%), indicating that all MEs using SAILs are as safe as conventional MEs. Therefore, the SAILs-based MEs represent potential low-toxicity alternatives to conventional Tween-80 ionic surfactant-based ME formulations.

3.4.7. Dissolution of Drugs in MEs

Four drugs, CLX, ACV, MTX and DTS, were used to evaluate the promising drug solubilizing or loading properties of the newly developed SAIL-based MEs as a delivery system for poorly soluble drugs. These drugs display poor solubility in water as well as most common organic solvents.[14,39] The presence of several polar groups in their structures could be attributed to their lower solubility in apolar solvent, even in a lipid/surfactant-rich mixture.[14,19,57] The solubility tests of drug molecules were conducted in water, 100% IPM, SAIL based IL/O MEs (SAIL[Cho][Ole] + Span-20/IPM/IL [C_1mim][$(CH_3O)_2PO_2$]) and conventional Tween-80 based IL/O MEs (Table 3.1).

Table 3.1. Solubility of drug molecules in SAIL [Cho][Ole] based ME systems at 25 °C

Systems	Surfactants mixture (%)	Drug solubility (mg/g)[a]			
		CLX	ACV	MTX	DTS
[Cho][Ole]-based ME[b]	10	10.92±0.76	10.15±1.20	3.46±0.23	3.67±0.43
[Cho][Ole]-based ME[c]	20	18.27±0.58	17.56±0.69	5.77±0.21	6.13±0.32
Tween-80-based ME[d]	20	3.93±0.34	3.54±0.41	–	–
Water	0	0.03±0.01	0.50±0.05	0.01±0.00	0.10±0.03
IPM	0	0.50±0.06	0.04±0.01	0.04±0.01	0.01±0.01

[a] The data represents the average of the three experiments.

[b] ME compositions are as follows: [surfactant mixture] = 10 wt.%, where the weight ratio of SAIL[Cho][Ole] to Span-20 was 2:1. [IL] = 3 wt.%. [IPM] = 87 wt.%.

[c] ME compositions are as follows: [surfactant mixture] = 20 wt.%, where the weight ratio of SAIL[Cho][Ole] to Span-20 was 2:1. [IL] = 5 wt.%. [IPM] = 75 wt.%.

[d] ME compositions are as follows: [surfactant mixture] = 20 wt.%, where the weight ratio of Tween-80 to Span-20 was 2:1. [IL] = 4 wt.%. [IPM] = 76 wt.%.

The solubility of the drug molecules (ACV) in newly developed IL/O MEs (20% total surfactant) was drastically enhanced by least 35-fold when compared with that of the

solubilities in water or IPM. The dissolution improvement in IL/O MEs may be attributed to the formation of hydrogen bonds between the anions of ILs and the polar groups of drug molecules.[14] The lower solubility of the drugs in IPM suggests the existence of drug molecules in hydrophilic IL cores, which are stabilized by creating an interfacial film between IPM and IL-based surfactant mixtures.[58] Drug loading in IL/O MEs increased by approximately two-fold when the concentration of surfactant mixtures was increased from 10 to 20% (Figure 3.7).

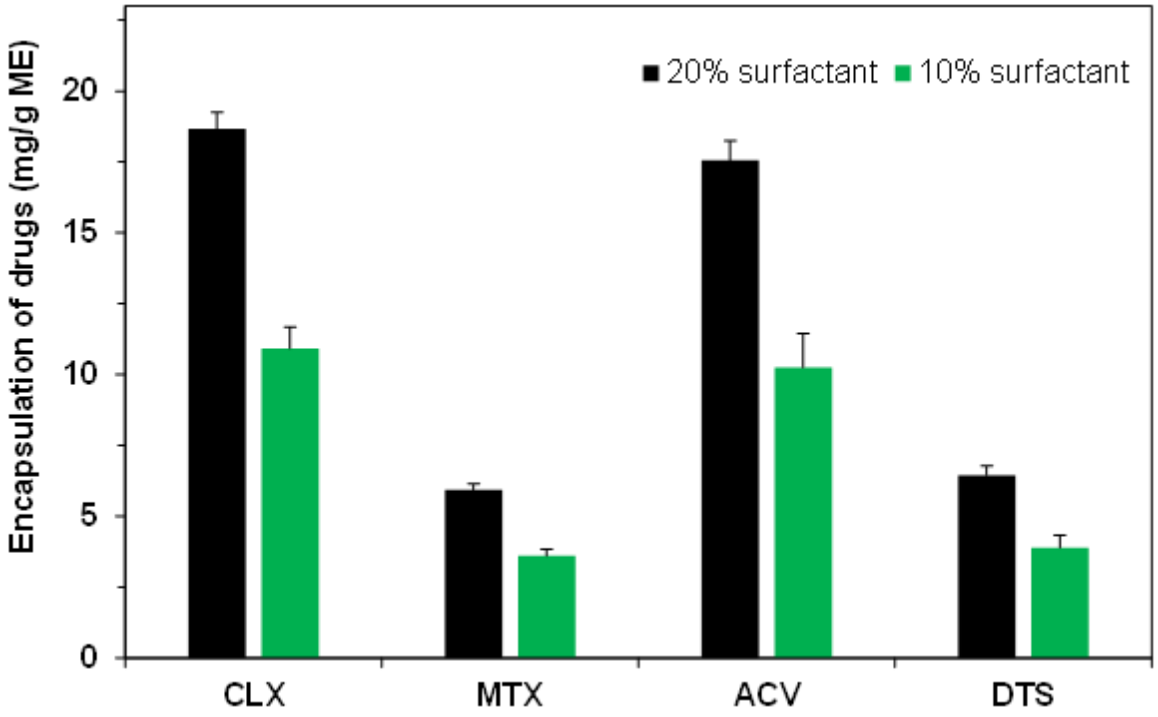

Figure 3.7. Dependence of drug solubility on the different total surfactant content used for formulation of MEs, where the ratio of SAIL[Cho][Ole] to Span-20 was 2:1.

By comparing these results with previous studies or conventional Tween-80 based IL/O MEs, the SAIL[Cho][Ole] based IL/O MEs showed higher drug loading capacity (Table 3.1). The possible reasons behind the higher solubilization and loading efficiency for SAIL-based MEs are the hydrogen bonding interaction between the positively charged group of cholinium ([Cho+]) and the electrodonating groups of the drugs.[59,60] In concert with H-bonding, π-type interactions are also play vital role to load drug molecules in the inner core of SAIL-based MEs, because of the electron rich conjugated π-system of the drugs molecule interact with electron deficient π system of the [Cho+], resulting the higher solubility of drugs as compared with conventional Tween 80 based MEs.[59,61–63] At 20% total surfactant mixtures, the SAIL based IL/O MEs showed 4.7 and 5-times higher loadings of CLX and ACV, respectively, when compared with that of the Tween-80 based IL/O MEs.[14] These results suggest that the newly

introduced SAIL based IL/O ME is a potential alternative to conventional surfactant-based MEs and could be used to develop an effective drug carrier for delivering poorly water-soluble drugs through transdermal or topical administration. The IL in the MEs may play a vital role in solubilizing the drugs and stabilizing the ME cores in IPM over long periods.

3.4.8. Stability of Drug Loaded MEs

The stability of drug-loaded MEs is very important in the design of promising IL/O MEs for effective drug delivery. The inherent stability of the micro-carrier containing drug molecules was investigated via visual observation and droplet size determination over 2 months at 25 °C. During storage (up to 120 d), the MEs exhibited no precipitation of drugs, color change, turbidity, phase separation and flocculation. As shown in Figure 3.8, the droplet sizes and size distributions of IL/O MEs were determined using DLS where no significant change of droplet size was observed after 50 d, indicating good physical stability of the drug-loaded IL/O ME system.[9,64] For example, the change in the droplet size for MEs containing 3.6 mg/mL CLX was from 13.7 (day 0) to 14.5 nm (day 50) (Figure 3.8(A)), whereas MEs containing 3.5 mg/mL ACV was from 11.9 to 17.5 nm over the same period (Figure 3.8(B)), thereby showing that no significant difference in droplet sizes occurred.

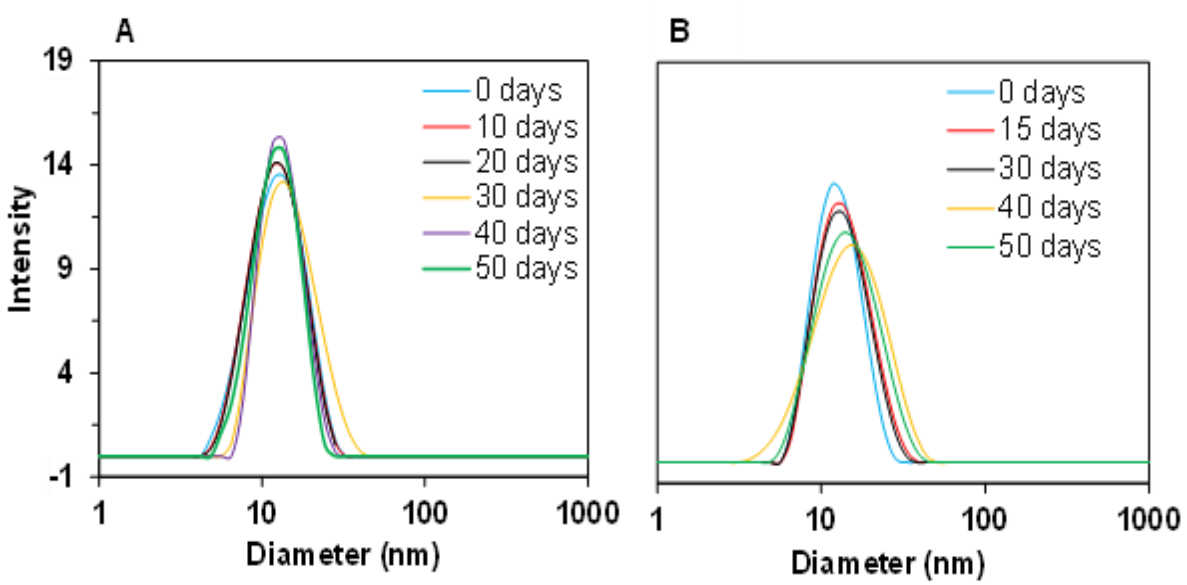

Figure 3.8. The hydrodynamic diameter of (A) CLX (3.6 mg/mL)- and (B) ACV(3.5 mg/mL) -loaded SAIL[Cho][Ole] based MEs at 25 °C. ME compositions are as follows: [surfactant mixture] = 20 wt.%, where the weight ratio of SAIL[Cho][Ole] to Span-20 was 2:1. [IL] = 4 wt.%. [IPM] = 76 wt.%.

These result indicated that SAIL surfactant-based MEs show similar stability when compared with that of conventional Tween-80 based MEs.[14,19] In addition, physical instabilities

such as aggregation, phase separation, phase inversion and cracking of the ME formulations were investigated through high centrifugation. No significant changes were visible observed, indicating excellent encapsulation of drugs at 25 °C.[65] The encapsulation efficiency of MEs (MEs contained 4 mg/mL of CLX and ACV initially) was found to be 97.1% and 94.5% for CLX and ACV, respectively, at the end of the storage period, indicating no degradation. The above results clearly show that SAIL surfactants are potential alternatives to conventional Tween-80 for ME-based drug delivery.

3.5. Conclusions

In this study, we reported novel cholinium fatty acid IL surfactant-based MEs with pharmaceutically accepted components, which have superior solubility with a series of insoluble or sparingly soluble drug molecules when compared with that of conventional Tween-80-based MEs. The pseudoternary phase diagrams clearly indicated that the formation of the larger ME region (two-fold higher) in SAIL[Cho][Ole] based-ME as compared with Tween-80 based-ME. DLS analysis of the prepared MEs with or without drugs clearly demonstrated excellent physico-chemical stability of the MEs and the presence of spherical micelle formation with diameters ranging from 6.5 to 21.2 nm. The cell viability of SAIL-based MEs (94%) was similar to Tween-80-based MEs (96%) at the same IL concentration (4%). At 20% total surfactant mixtures, the SAIL based IL/O MEs showed 4.7 and 5-times higher loadings of CLX and ACV, respectively, when compared with that of the Tween-80 based IL/O MEs. The significant solubility enhancement of these drugs using this IL/O MEs is most likely because of the formation of hydrogen bonds and electrostatic interactions among the head groups of SAIL surfactants, the anions of ILs and the polar groups of drug molecules. The results will undoubtedly change the perception of conventional Tween-80-based ME formulations and the introduced SAIL-based MEs represent promising target drug delivery systems. Nonetheless, further work is required to characterize the mechanisms of drug delivery into skin.

3.6. References

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CHAPTER 4: SUMMARY AND FUTURE WORK

4.1 Summary

During the last several years, ILs have been at the top of the most promising research materials and technology in the scientific fields. ILs are not only applicable to be covered the classical areas of knowledge but also expended as an important candidate to solve critical problems such as clean and efficient energy. In the pharmaceutical industries, the researchers are facing unparalleled challenges to develop the effective drug delivery systems for achieving targeted pharmacological response of many drugs because of their polymorphism, limited solubility, permeability, and bioavailability. To address these limitations, an IL based formulation is a promising approach to design the smart delivery of drugs. Particularly ILs were found to play a unique role in the pharmaceutical industry as clean and multifunctional properties for a variety of applications. This neoteric class of solvents, ILs, generally present interesting properties, such as negligible vapor pressure at relatively ambient conditions, high thermal, chemical, high surface activity and electrochemical stability, and broadly tunable properties with regard to polarity, hydrophobicity, and solvent miscibility. One of the major challenges in the pharmaceutical research is the increasing number of active pharmaceutical ingredients (APIs) possessing limited aqueous solubility in the biopharmaceuticals classification system class II drugs. In last few years, ILs have been considered as solvents and/or materials in the field of pharmaceuticals with the possibility of improved performance such as improved solubility, stability, and drug delivery. In the last decade, ionic liquid (ILs)-based microemulsions (MEs) have been tested as potential nanocarriers for TDD. ILs are tunable designer solvents and play a versatile role at all phases of ME systems by altering water, oil and surfactant components. Recently, researchers have introduced ILs as a surface-active IL (SAIL) to serve as a surfactant by combining cations and anions with long alkyl chains for formulating different organized assemblies and improving the physico-thermal stabilities of MEs over aqueous conventional systems. In this thesis, we mainly focused on the synthesis and characterization of surface-active ionic liquids (SAILs) and evaluated their feasibility for biomedical applications.

In **chapter-2,** we preliminary reported a series of potentially low toxic and halogen free choline–fatty-acid-based surface-active ionic liquids (SAILs) were synthesized and their aggregation behavior in aqueous medium was evaluated. A series of fatty acids such as oleic acid, linoleic acid and erucic acid were selected as anions and cholinium as the cation because of their low toxicity and biocompatibility. All synthesized SAILs were highly viscous with

high yield and purities (> 97.0%). Then all the SAILs were characterized using ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy, Fourier transform infrared spectroscopy, differential scanning calorimetry, and elemental analyses. The surface-active properties of the SAILs were investigated by tensiometry, conductometry, and dynamic light scattering measurements.The critical micelle concentration (CMC) values for the newly synthesized SAILs in water were found to be 2 to 4 times lower than those of the conventional ionic surfactants, SDS and SDBS because of the presence of a longer hydrophobic alkyl chain or/and the size difference of the cations (Cho^{+} and Na^{+}), which are less hydrated by water and provide the strong electrostatic repulsion between the charged head groups in the surface layer. In addition, the thermodynamic properties of micellization (ΔG^{0}_{m}, ΔH^{0}_{m}, and ΔS^{0}_{m}) indicated that the micellization process of the SAILs is spontaneous, exothermic, and entropy-driven at room temperature. Then the formation of spherical micelles with a range of 3.9-5.6 nm in diameter was determined by the dynamic light scattering measurement. The cytotoxicity of the SAILs was evaluated using mammalian cell line NIH 3T3. Moreover, the toxicity profile of [Cho][Ole] falls in the range of almost harmless compared to that of Tween 80. In addition, [Cho][Eru] is more toxic than [Cho][Ole], which is less toxic than some traditional surfactants. Finally, we believe that this environmentally friendly SAILs can be used as potential alternative to conventional surfactants for various fields, including biological and biomedical applications.

In **chapter-3,** as a follow-up to previous study, we reported novel cholinium fatty acid IL surfactant-based microemulsions (MEs) with pharmaceutically acceptable components, which have superior solubility with a series of insoluble or sparingly soluble drug molecules when compared with that of conventional Tween-80-based MEs. In this study, a series of SAILs was formulated a novel IL/O MEs by a mixture of SAIL, sorbitan laurate (Span-20) and isopropyl myristate. Based on the constructed pseudo-ternary diagrams, SAILs played the vital role as a surfactant while Span-20 acted as the co-surfactant, showing excellent solubility of the drug into the MEs system prepared with a ratio of 2:1 (SAIL[Cho][Ole]: Span-20). The droplet shape, size and size distribution of MEs were investigated through dynamic light scattering and found well-distributed particle size in the range of 6.5 to 21.2 nm, demonstrating the spherical micelles formation and the presence of IL is in the core of MEs. In order to explore the MEs as a potential drug carrier, we have investigated the solubility of some sparingly soluble drug molecules (e.g., celecoxib, acyclovir, methotrexate and dantrolene sodium) into the MEs system and found excellent solubility of such drugs in the ME system as compared with conventional tween-80 surfactant-based ME because of the formation of hydrogen bonds and

electrostatic interactions among the head groups of SAIL surfactants, the anions of ILs and the polar groups of drug molecules. Then the inherent stability of the micro-carrier containing drug molecules was investigated via visual observation and droplet size determination over 2 months at 25 °C. During the storage (up to 120 d), the MEs exhibited no precipitation of drugs, color change, turbidity, phase separation and flocculation. Finally, in vitro cytotoxicity of the new carrier was evaluated using 3-dimensional reconstructed human epidermis model and found the cell viability of SAIL based MEs (94%) was almost similar as compared with tween-80 based MEs (96%) at the same IL concentration (4%). The results will undoubtedly change the perception of conventional Tween-80-based ME formulations and the introduced SAIL-based MEs represent promising target drug delivery systems.

In conclusion, the obtained results (**chapter 2 & 3**) suggest that SAILs could be a potent biocompatible surfactants to eliminate the use of traditional toxic surfactants for oral/ topical/ transdermal delivery of poorly water-soluble drugs, since they allow an improved solubility of poorly water-soluble drugs.

4.2 Future work

As highlighted in the present studies, SAILs will be used as promising alternatives for conventional surfactants due to their superior surface activity. We also believe that this work would open new possibilities for the development of IL-based biocompatible surfactants for various fields, including biological and biomedical applications. However, newly synthesized SAILs would be a potential surfactant in the IL-based microemulsion system in the drug delivery for poorly water-soluble drugs although their molecular mechanisms are yet explored. The study will undoubtedly change the perception of conventional surfactant-based ME formulations and the introduced SAIL-based MEs represent promising target drug delivery systems. Nonetheless, further work is required to characterize the mechanism of the drug delivery into skin.

However, the development of pharmaceutical applications using IL-based formulations requires a deep understanding of ILs both in terms of their macroscopic properties and also at the molecular level, because the structural aspects have been shown to play a crucial and unexpected role in a large number of situations. Additional consideration should be concentrated on the large-scale production of biocompatible and biodegradable ILs. Research into these issues presented here may be regarded as a starting point for this novel formulation

platform, leading to a better insight of ILs as a formulation strategy and promising new IL based drug delivery systems.

ABBREVIATIONS

ILs	Ionic liquids
SAIL	Surface-active ionic liquid
Cho	Choline
Ole	Oleate
Lin	Linoleate
Eru	Erucate
DMSO	Dimethyl sulphoxide
CMC	Critical micelle concentration
DSC	Differential scanning calorimetry
SDS	Sodium dodecyl sulfate
SDBS	Sodium dodecyl benzene sulfate
API	Active pharmaceutical ingredient
API-IL	Active pharmaceutical ingredient ionic liquid
FDA	Food and drug administration
GRAS	Generally regarded as safe
GIT	Gastrointestinal tract
PBS	Dulbecco's phosphate buffered saline
SIF	Simulated intestinal fluid
SFG	Simulated gastric fluid
MEM	Minimum essential media
TDD	Topical or transdermal drug delivery
SC	Stratum corneum
MEs	Microemulsions
AAE	Amino acid ester
IC_{50}	Half maximal inhibitory concentration
LD_{50}	Half maximal lethal dose
MTX	Methotrexate
ACV	Acyclovir
CLX	Celecoxib
DSC	Differential scanning calorimetric
NMR	Nuclear magnetic resonance spectroscopy
FT-IR	Fourier transform infrared

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Figure 3.7. Dependence of drug solubility on the different total surfactant content used for formulation of MEs, where the ratio of SAIL[Cho][Ole] to Span-20 was 2:1.

Figure 3.8. The hydrodynamic diameter of (A) CLX (3.6 mg/mL)- and (B) ACV(3.5 mg/mL) -loaded SAIL[Cho][Ole] based MEs at 25 °C. ME compositions are as follows: [surfactant mixture] = 20 wt.%, where the weight ratio of SAIL[Cho][Ole] to Span-20 was 2:1. [IL] = 4 wt.%. [IPM] = 76 wt.%.

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