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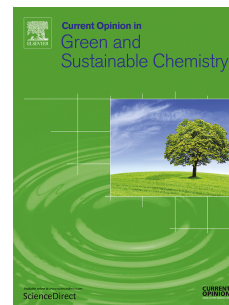
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**Amphiphilic ionic liquids as sustainable components to formulate promising vesicles
to be used in nanomedicine**

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Abstract

Ionic Liquids (ILs) are an interesting category of compounds particularly due to the possibility to easily synthesize them, with different properties in the lab. The interest for ILs with amphiphilic character (IL-like surfactants) has been growing up due to versatility in creating useful supramolecular assemblies. In this short review, it is described the current state of IL-like surfactants to prepare vesicles in water. Particularly, the focus was put on the experiments performed by South American researchers using protic and aprotic surfactants. Finally, it summarizes some preliminary results obtained in our lab about the application in nanomedicine that these novel vesicles can offer.

Introduction

When amphiphilic molecules are molecularly dispersed in water, the polar part of the amphiphile tends to expose itself to bulk water while the hydrophobic part shields itself from the aqueous phase. Depending on the amphiphile structure and concentration, different types of assembly may form spontaneously. Some lipid structures such as phospholipids form bilayer structures.[1] Mostly, the phospholipid-like amphiphiles form double-layer structures (lipid bilayers) by contacting the hydrophobic faces of two leaf-like monolayer amphiphile assemblies. This lipid bilayer structure extends two-dimensionally and forms the covering of a closed sphere that has an internal water environment, similarly to a cytomembrane in nature, of a closed sphere that has a water pool inside. Thus capsule-like structure can be considered a simplified model of a cell, usually called *liposome* (Figure 1). Several other synthetic amphiphiles can form a liposome-like supramolecular assembly. Those derived from double-tailed cationic surfactants are usually called vesicles, while the term liposome is related to phospholipid assemblies.[1] Vesicles prepared from cationic surfactants have advantages such as simple formulations and greater stability to hydrolytic and oxidative degradation. Since the discovery of this self-assembly phenomenon in the 1960s, lipid-based self-assembled nanostructures have been widely studied and applied in drug delivery systems.[2] Thus, a variety of vesicular systems can be designed, either to deliver hydrophilic molecules encapsulated in the water core or hydrophobic drugs integrated to the lipid bilayer.

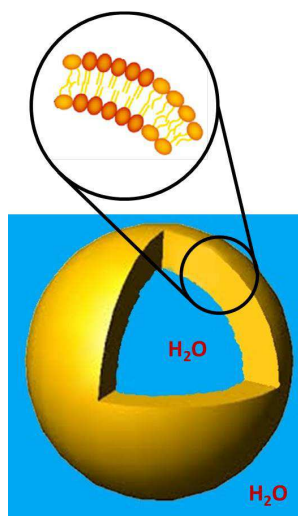


Figure 1. Liposome with a single bilayer.

It is worthy to mention here that, upon solubilization in water the phospholipid bilayer first exists as a lamella (a flat plate-like structure) before getting converted into spheres. Several lamellae of phospholipids bilayers are stacked one on top of the other during the formation of liposomes to form a multilamellar structure requiring different methods to be transformed into unilamellar vesicles, which is the kind of system suitable for physicochemical studies and/or usage as a drug delivery device for living organisms.[3,4] Hence, they are not easy to prepare from phospholipids and generally have limited stability and shelf-life duration.

It is known that ionic liquids (ILs) are a powerful alternative to conventional materials by the principles of Green Chemistry because they have negligible vapor pressure combined with excellent chemical and thermal stability, ease of recyclability and widely tunable properties such as polarity, hydrophobicity, and solvent miscibility through appropriate modification of the cation and anion.[5] Although with some disadvantages, [6] these properties make ILs potentially environmentally friendly media for many chemical

processes. Hence, it is quite interesting to synthesize them [7–10] with amphiphilic properties.[11,12] These compounds, called *IL-like surfactants*, have been used to create different kinds of organized systems such as direct micelles, Reverse Micelles (RMs), and vesicles.[8,10,13–18] In the last decade, it has been explored the use of a particular type of IL-like surfactant as a possible candidate to form vesicles in water: catanionic surfactants, which are a fairly new kind of molecules with very motivating properties as amphiphiles.[10] These compounds are the result of the mixture of oppositely charged surfactants. In the literature, two categories of catanionic surfactants can be found: i) catanionic mixtures, which are simple mixtures of anionic and cationic surfactants without the removal of their respective counterions; ii) catanionic systems, which correspond to the mixed surfactants where the counterions are removed.[10,19,20] The usual way to obtain catanionic systems is by the combination of two opposite charged ionic surfactants, in a 1:1 molar ratio, followed by the removal of the counterions using an appropriate method.[10] The amphiphiles thus obtained have shown the ability to form different organized systems, such as direct and RMs[10,21,22], vesicles[10,23], and liquid crystals.[24] Particularly, some unilamellar vesicles have been synthesized with catanionic surfactants and showed higher stability when compared to liposomes.[2,25] When the catanionic surfactant, BHD-AOT (Scheme 1) was synthesized in the lab for the first time it was found that met all properties of an IL. The catanionic surfactant BHD-AOT, prepared from an equimolar mixture of the anionic surfactant [26] sodium 1,4-bis-2-ethylhexylsulfococinate (Na-AOT) and the cationic surfactant benzyl-n-hexadecyldimethylammonium chloride (BHDC), for instance, showed IL-like properties. Dynamic Light Scattering (DLS) results showed that the size of the BHD-AOT aggregates about 80 nm [10] is comparable to the size of vesicles.[27–30] Small Angle X-Ray Scattering (SAXS) results showed that a vesicle

model with a single diffuse lamellar shell is the one that better describes those aggregates, indicating that unilamellar vesicles of BHD-AOT [10] were formed in water, without the addition of energy to the system, as required by traditional methods.

Results of emission spectroscopy, using two different probes (6-propionyl-2-dimethylaminonaphthalene – PRODAN – and trans-4-[4-(dimethylamino)-styryl]-1-methylpyridinium iodide – HC) showed unique properties of BHD-AOT bilayer in comparison with vesicles formed using the traditional phospholipid 1,2-di-oleoyl-sn-glycero-3-phosphatidylcholine (DOPC)[31], like a powerful electron donor environment. Additionally, the BHD-AOT bilayer offers a less polar and slightly more viscous medium than DOPC, which allows large incorporation of ionic and nonionic molecules.

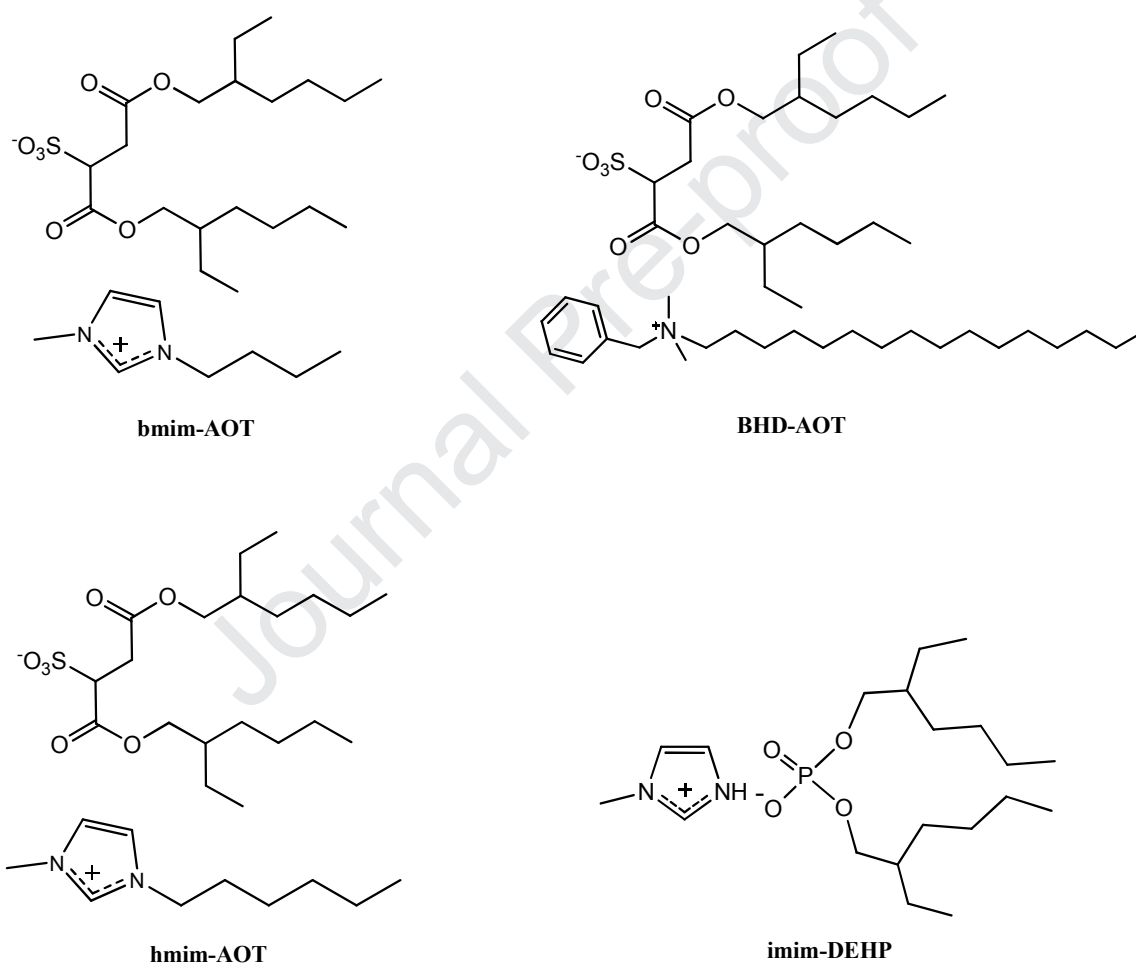
Concerning IL-like surfactants long-chained imidazolium, ILs are the more conventional molecules used, including a charged hydrophilic head group and one or more hydrophobic tails.[11,12,40,41,32–39] Depending on the hydrogen bond donor ability of the components these ILs can be classified as protic and aprotic.[42]

The aprotic IL 1-butyl-3-methylimidazolium 1,4-bis-2-ethylhexylsulfosuccinate (bmim-AOT, Scheme 1), is an interesting case of IL-like surfactant which has been used as surfactant to create RMs.[16] Even the behavior of this surfactant in aqueous media was previously reported [11,43], although it is not clear which kind of aggregates are formed in water. For example, at low concentrations, when interactions between aggregates are minimized, their behavior was not evaluated.

Recently Garcia et al.[44] reported the spontaneous formation of vesicles in an aqueous solution of cationic mixtures composed of different IL-like surfactants, methylimidazolium- or pyridinium-based, non-functionalized or bearing an ester or amide group in the alkyl chain, and the anionic surfactant Na-AOT. They showed that the

catanionic mixtures studied exhibit high antimicrobial activity against fungi and most Gram-positive bacteria and low toxicity to Gram-negative microorganisms. These properties could be of special interest for their application in nanotechnology and pharmaceutical formulations.

In this review, recent studies, performed by South-American researchers, involving the formation of vesicles using different amphiphilic ILs are described.



Scheme 1. Chemical structure of different amphiphilic ILs.

Current status

Vaid et al. [40] investigated the effects of a well-known anti-inflammatory drug (diclofenac sodium, DS) on the morphology of aqueous micellar aggregates of two ILs-like surfactants: 1-hexadecyl-3-vinylimidazoliumbromide and 1-hexadecyl-3-methylimidazolium bromide, for potential therapeutic use as drug delivery systems in topical applications. They employed several techniques such as turbidity, viscosity, DLS, and transmission electron microscopy (TEM). They found that the DS-induced morphological changes of the micellar aggregates depend on the concentration of DS and the structure of the surfactant head-group. The observed transitions from spherical micelles/worm-like micelles/vesicles are considered as due to the interaction between the anionic diclofenac and the imidazolium ring. The changes in morphology agree with the expected effects of DS on the packing parameter of the aggregates. They also studied that cholesterol induces the transition to unilamellar vesicle formed in aqueous medium with the functionalized and nonfunctionalized ILs based surfactants named before and compared with cetyltrimethylammonium bromide.[41] They explore the effect of cholesterol concentration on the surfactant solution and determine the optimal concentration which provides unilamellar vesicles. They show for the first time that cholesterol can induce vesicle formation in the functionalized surfactant, 1-hexadecyl-3-vinylimidazolium bromide. This surfactant displays micellar transition at a lower concentration of the cholesterol and forms more rigid and hydrophobic bilayer among the studied amphiphiles. The vesicles obtained with the three surfactants show great stability with time, temperature, and dilution with water. Additionally, Vaid et al. [39] studied salt-induced vesicle formation in the same functionalized and vinyl functionalized ILs-like surfactants to obtain insight into which are the factors that influence the structure of the formed aggregates. Electrostatic attraction, hydrophobic interaction, cation- π and π - π interactions along with

H-bonding interaction between the aromatic anions of the salt and ILs based surfactant monomers are the major factors, which govern the formation of larger aggregates for these compare to cationic conventional surfactants.

Like previously mentioned, IL bmim-AOT can form RMs.[16] Recently, the ability of bmim-AOT to form aggregates in dilute aqueous solution was evaluated, and to investigate the effect of counterions on the properties of the formed aggregates, 1-hexyl-3-methylimidazolium 1,4-bis-2-ethylhexylsulfosuccinate (hmim-AOT, Scheme 1) was also investigated.[45] Both the ILs were very soluble in water, and opalescent solutions stable over time were obtained, suggesting the formation of large aggregates.[46] By DLS, diameters ranging from 146 - 164 nm for bmim-AOT and, from 93 - 120 nm for hmim-AOT, with low polydispersity indexes were detected. These size values were independent of the surfactant concentration being good evidence that the aggregates spontaneously formed are vesicles and not direct micelles.[11,30,43,47–50] The negative zeta potential values showed in both vesicles (bmim-AOT: -21 mV and hmim-AOT: -16 mV), suggests that the anionic part of the amphiphile is exposed to the outer region of the vesicular bilayer. Moreover, the low polydispersity index values, indicate that they are unilamellar vesicles.[9,10] These results are very interesting because the precursor surfactant (Na-AOT) of both ILs cannot form spontaneously unilamellar vesicles in water at the surfactant concentrations evaluated.[30,43,47–51] On the other hand, several reports have shown data about the vesicle formation with Na-AOT but use different methodologies to prepare them (such as extrusion).[52–57] Therefore, the chemical structure of the cationic components in these new surfactants strongly impacts their aggregation process.[11,43,57] As an interesting application, both vesicles were combined with DNA showing that the length of the hydrophobic chains of the counterions influences the DNA-surfactant interactions.[45]

Circular dichroism experiments were performed to investigate the interactions between DNA and the vesicles. The spectra show that the vesicles interact with the DNA partially modifying the polynucleotide conformation. These results deserve further investigation evidence that these vesicles are prone to be used in DNA extraction, gene therapy, and gene transfection.

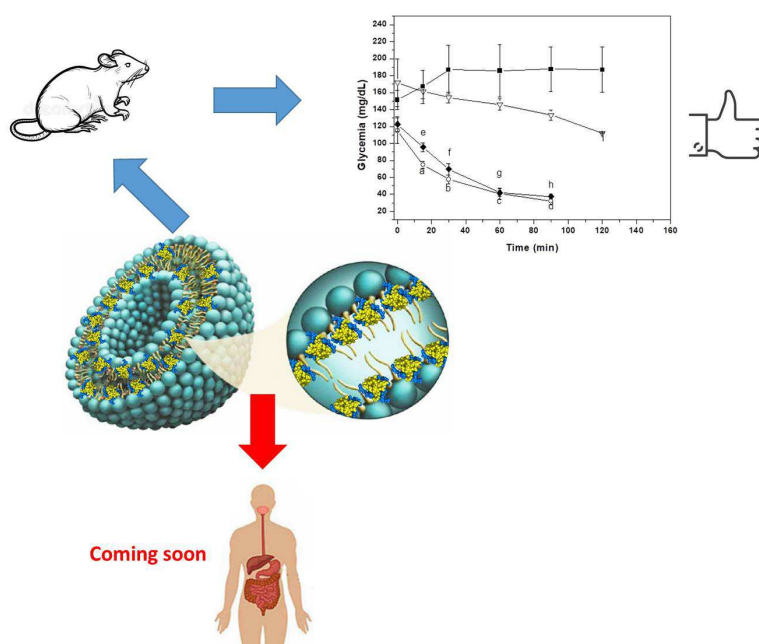
Taking into account the versatility that the ILs synthesis offers, a protic IL with amphiphilic character (1-methylimidazolium bis-(2-ethylhexyl)phosphate - imim-DEHP, Scheme 1) was synthesized by the acid-base reaction between bis-(2-ethylhexyl)phosphoric acid (HDEHP) and 1-methylimidazole.[9] Imim-DEHP demonstrated to form RMs in no polar solvents.[9,58] Moreover, it forms spherical aggregates in water with diameters ranging from 120-140 nm with a low polydispersity index (0.1-0.3) as measured by DLS and TEM techniques. Furthermore, all the data are consistent with the spontaneous formation of unilamellar vesicles. Formation of aggregates at low concentrations (10^{-5} M) and the detection of aggregates larger than a few nanometers,[51,59,60] suggest that the formed structures are probably vesicles and not direct micelles. Additionally, these vesicles have a zeta potential values around -46 mV at the different surfactant concentrations explored, suggesting that anionic moieties are exposed to the outer face of the vesicle bilayer. These facts are promising since sodium bis-(2-ethylhexyl) phosphate (Na-DEHP), which is difficult to synthesize, forms in water multilamellar aggregates with a diameter around 50 nm.[61,62] Additionally, HDEHP does not present the ability to form vesicles in water, denoting that the chemical structure of the cationic component in the new surfactant impact strongly in its aggregation process.[43]

Thus, the easy method to form imim-DEHP and its preliminary properties make it an interesting candidate for drug delivery applications.

In comparison with traditional phospholipids such as DOPC, the catanionic surfactant BHD-AOT forms spontaneously unilamellar vesicles with distinctive properties, such as polarity and viscosity of the bilayer.[10,31] As a complementary exploration, it has been evaluated the permeability (P) of the bilayer of BHD-AOT vesicles. The coefficient P-value was measured using a non-traditional technique such as the electrochemistry and a solute model: 1-naphthyl phosphate (1-NP).[63] Thus, 1-NP was added in the external aqueous phase of the vesicle with the encapsulated enzyme (alkaline phosphatase). To react with the enzyme, 1-NP must cross the vesicle bilayer yielding 1-naphtholate, which was detected by a square wave voltammetry. A value of $P = (1.00 \pm 0.15) \times 10^{-9} \text{ cm s}^{-1}$ was obtained, which is comparable, for example to P values of DOPC vesicles.[63] Thus, electrochemical detection proved to be a good alternative to measure the permeability of vesicle solutions, in which the turbidity of the sample makes the use of spectroscopic methods more difficult.

Taking into account our previous characterization of BHD-AOT vesicles and, to use them as drug delivery agents, they were evaluated under different pH conditions.[64] The diameter values of the BHD-AOT vesicles determined by DLS at different times at low pHs values ($\text{pH} \leq 2$) to simulate stomach like conditions show that the vesicles are stable during about 90 minutes, after that, the size value decreases sharply to almost half of the original size. Moreover, during the first 90 minutes of exposition, the polydispersity reminds almost constant and then increases abruptly. Interestingly, even that the BHD-AOT vesicles turn unstable under acidic conditions at longer times, which is one remarkable difference when compared to the vesicles obtained using traditional phospholipids, which do not resist the

low pH values and collapse due to hydrolysis.[65] The BHD-AOT chemical structure (see Scheme 1), do not seem to suffer the same lysis process that DOPC vesicles in acidic environments ($\text{pH} \leq 2$). The results of *in vitro* (globular resistance, MTT, trypan blue), *in vivo* (Dose lethal 50 and toxicity chronic) and stomach like pH simulation studies, demonstrate that they are safe for living systems due to the low toxicity observed in doses lower than 0.05 mg/mL *in vitro* or 118.6 mg/Kg *in vivo*. This characteristic makes the BHD-AOT vesicle highly biocompatible and a very capable candidate for oral drug delivery. In this sense, very recently [66] it was demonstrated that BHD-AOT vesicles showed appropriate properties to encapsulate and protect insulin. Moreover, *in vivo* assays in mice show a significant decrease in glycemia after oral administration (Scheme 2). The described results confirm that BHD-AOT vesicles are a potential system to be administrated in different ways (oral, for example) as an alternative strategy to conventional therapy.



Scheme 2. BHD-AOT vesicles used to deliver insulin. Graph adapted from reference [66].

Conclusions and perspectives

In this short review, it has been shown works that demonstrate how amphiphilic ILs, can be used to generate easily unilamellar vesicles in aqueous solutions. They are formed spontaneously and do not require additives or extra energy. They are stable in neutral conditions. Their bilayer has been characterized by a variety of methods including electrochemical method that was developed to measure their permeability and an electrochemical method was developed to measure their permeability. Moreover, it is important to emphasize that the amphiphilic ILs are no more expensive than a common IL or conventional surfactant retaining all the sustainable properties of ILs. These studies are very promising since vesicles are of technological interest for many applications such as drug delivery, bioseparations, and sensing and maybe good substitutes for liposomes.

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Abbreviations

1-NP: 1-naphthyl phosphate.

BHD-AOT: benzyl-n-hexadecyldimethylammonium 1,4-bis-2-ethylhexylsulfocinate.

BHDC: benzyl-n-hexadecyldimethylammonium chloride.

bmim-AOT: 1-butyl-3-methylimidazolium 1,4-bis-2-ethylhexylsulfosuccinate.

DLS: Dynamic Light Scattering.

DOPC: 1,2-di-oleoyl-sn- glycerol-3-phosphatidylcholine.

DS: Diclofenac Sodium.

HC: trans-4-[4-(dimethylamino)-styryl]-1-methylpyridinium iodide.

HDEHP: bis-(2-ethylhexyl)phosphoric acid.

hmim-AOT: 1-hexyl-3-methylimidazolium 1,4-bis-2-ethylhexylsulfosuccinate.

ILs: Ionic Liquids.

imim-DEHP: 1-methylimidazolium bis-(2-ethylhexyl)phosphate.

MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

Na-AOT: Sodium 1,4-bis-2-ethylhexylsulfocinate.

Na-DEHP: sodium bis-(2-ethylhexyl) phosphate.

P: permeability.

PRODAN: 6-propionyl-2-dimethylaminonaphthalene.

RMs: Reverse Micelles.

SAXS: Small Angle X-Ray Scattering.

TEM: Transmission electron microscopy.

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The authors synthesize for the first time a novel protic surfactant which is an ionic liquid able to form reverse micelles and vesicles. This is a novel ionic liquid-like surfactant derived from dialkyl phosphate.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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