Supported Lonic Liquids Used as Chromatographic Matrices

Subjects: Others

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Ionic liquids (ILs) have been investigated as novel ligands in chromatographic matrices, denominated as supported ionic liquids (SILs). ILs are organic salts with a wide structural diversity, which can display a multi-modal behavior because they present positive/negative charged groups, and can be tailored by the introduction of several functional groups and alkyl moieties of different lengths. SILs maintain the valuable features of ILs with the addition of being supported, thus avoiding the use of large amounts of ILs. Despite the fact that the liquid state of ILs is being lost when immobilized, their capability to establish a plethora of interactions is kept, allowing them to be used in hydrophilic, hydrophobic, affinity, multi-modal and ion-exchange chromatography. Due to their advantages, IL-modified materials have been recently synthetized and proven to be an important new type of stationary phases in liquid chromatography.

Keywords: biomolecules ; chromatographic supports ; ionic liquids ; ligands immobilization ; selectivity

1. Introduction

Liquid chromatography is one of the most widely used methods in the field of biotechnology, both at the analytical and preparative levels. High-performance liquid chromatography (HPLC) consists of a chromatographic approach to separate, identify, and quantify specific components of a given mixture, for example, for the identification of constituents of a biological sample or separation of chemical compounds [1]. This technique is the method of choice for analytical procedures, and well-established operation modes, types, and stationary phases are recognized [2]. On the other hand, preparative chromatography consists of the effective purification of high-value products from complex mixtures [3]. Both types of chromatographic approaches rely on the distribution of a target molecule between the mobile and stationary phases. The performance of the preparative chromatography highly depends on the selection of efficient stationary phases with great selectivity towards the molecule of interest [3][4][5]. Initially, supports were designed for the separation of small molecules; however, with the increased demand for protein purification, novel matrices with enhanced robustness and selectivity were developed [6]. More recently, with the advances of nucleic acid-based therapeutics, new challenges are imposed on the purification strategies. Thus, novel adaptations to the chromatographic supports are required, and specific interactions are mandatory for developing effective chromatographic methods for the purification of these high-value biomolecules [7]. The techniques currently available to isolate and purify nucleic acids still present several limitations, enhancing the necessity of establishing new methods able to improve biomolecules' quality to fulfill the requirements of the regulatory agencies. Amongst these purification processes, chromatography still remains the technique of choice due to its enhanced performance to achieve improved selectivity towards the target biomolecule [8]. Conventional purification protocols based on precipitation with salts, temperature, pH, and high molecular weight polymers have been replaced by highly selective and revolutionary strategies, such as affinity chromatography [9][10][11]; multi-modal chromatography occupies an important place in the bioseparation field, once it adds a new dimension to conventional chromatography procedures, such as ion-exchange, hydrophobic interaction, reversed-phase, or affinity [12][13]. Usually, multi-modal chromatographic matrices present multiple functional groups that cooperate in binding and elution steps. These groups offer the possibility of various interactions to occur, such as hydrophobic, aromatic, electrostatic, and hydrogen-bonding interactions. Initially, multi-modal matrices were composed of aliphatic hydrophobic groups and were mainly used for the extraction and purification of small organic compounds. These were not useful for biomolecules once they became strongly bound, and the elution required the application of organic solvents. Lately, new versions of multi-modal matrices composed of aromatic and charged groups have appeared in which interactions with the target product are not so strong and can be disfavored using mild conditions such as increased/decreased salt concentrations or changes in pH [14].

Amongst the possible ligands in chromatographic matrices, in recent years, ionic liquids (ILs) have been proposed to functionalize stationary phases, giving rise to supported ionic liquids (SILs) [15][16]. ILs are organic salts with a wide

structural diversity, which can display a multi-modal behavior because they present positive/negative charged groups and can be tailored by the introduction of several functional groups and alkyl moieties of different lengths [15][16].

2. Ionic Liquids

In the past, ILs have attracted the attention of many researchers due to their versatility and possible application in several areas, particularly emphasizing the potential to develop greener and sustainable processes [17][18]. The first ionic liquid (IL) was discovered by Paul Walden in 1914 when searching for molten salts that were liquid at temperatures at which his equipment could be used without special adaptations. Walden's interest in these molten salts was the relation of their molecular size and their conductivity; however, the potential of this breakthrough went unnoticed for a long time. Almost 40 years later, the potential benefits of the lower melting points of molten salts were deeply recognized [19]. Today, ILs present a wide range of applications in various fields, such as organic, inorganic, physical, and biological chemistry [18][20].

Since there are numerous possible cation and anion combinations, allowing the design of task-specific ILs, ILs are recognized as "Designer Solvents" [21][22]. Due to their ionic character, most ILs (if properly designed) present some outstanding features, including low volatility, non-flammability, variable viscosity and ionic conductivity, wide electrochemical potential window, high solvation ability, and excellent chemical, thermal, and electrochemical stability [21][22][23]. The first two characteristics contributed to the classification of ILs as "Green Solvents", and, as a result, these compounds have been viewed as good alternatives to replace volatile organic solvents (VOSs) recurrently used in a wide range of processes. Ideally, this replacement would eliminate the loss of these solvents to the atmosphere and consequently reduce the harmful effects to the environment and human resources, making it possible to develop "greener" processes [22][24]. Nevertheless, other characteristics of ILs need to be taken into account before such claims can be made, such as their eco/citotoxicity and biodegradability. ILs are also usually recognized by their excellent solvation capacity for a wide range of compounds, as well as good stabilizing media for proteins, nucleic acids, and other bioproducts [21].

Besides being used as solvents, most of the time in their neat form or in aqueous solutions, ILs can be used in chromatography as ligands of the stationary phase, which is the main focus of this research [16]. With this, it is possible to combine the most powerful chromatography technique for biomolecules purification with the outstanding characteristics of ILs. Nevertheless, it should be noted that once bound to a solid support, the cation/anion pair no longer constitutes a true IL. However, one of the most interesting properties of classic ILs, that is, their tunability, is maintained even when attached to chromatographic supports. The morphology of immobilized ILs varies, but the characteristics that depend on the structure of cation or anion can be preserved, ensuring the possibility to promote multiple interactions, such as hydrophobic, electrostatic, dipole–dipole, π–π, and hydrogen bonding [25]. SILs maintain the valuable features of ILs with the addition of being supported, thus avoiding the use of large amounts of ILs, which can be costly and further prevent some toxicological concerns [26].

2.1. Supported Ionic Liquids (SILs) in Analytical Methods

ILs have been applied in several techniques, including extraction, chromatography, and spectroscopy. The growing interest of ILs in analytical chemistry is testified to by the increased number of publications that appeared during the last decade [27][28][29]. It should be mentioned that an impressive amount of works aimed to develop new stationary phases for HPLC, in order to improve column efficiency, permeability, and stability [30]. Reports concerning the applications of SILs have mainly focused on silica functionalization for gas chromatography [31]. Additionally, applications of ILs as stationary phases for hydrophilic interaction chromatography (HILIC) separations started to emerge [32]. In this entry, the researchers will focus on the use of ILs in solid supports for biomolecules separation and purification by means of liquid chromatography.

In the last years, advances towards the covalent immobilization of ILs onto silica materials and in the attachment of ILs onto polymers have been faced [31]. ILs can be immobilized in different ways, but always require the establishment of interactions between the ionic liquid or its components (anions or cations) and the support material or functional group (or active species) [33].

IL-based supports received relevant attention due to their excellent properties and potential application in many fields of analytical chemistry. IL-modified polymers constitute better alternatives to the functional porous polymers, such as Sepharose and other commonly used copolymers, that have been used as stationary phase in HPLC separation once, besides achieving great selectivity, they also offer increased column efficiency [34]. More detailed information about stationary phases already used in HPLC can be found in the works of Vidal et al. [31] and Pino and Afonso [30].

2.2. ILs as Ligands of Chromatographic Supports

Usually, when considering liquid chromatography, ILs are immobilized on the surface of solid supports by covalent bonding of their cations or anions [16], which is highly relevant to avoid the IL leaching. There are six ways to perform the IL immobilization onto the support, as shown in **Figure 1**.

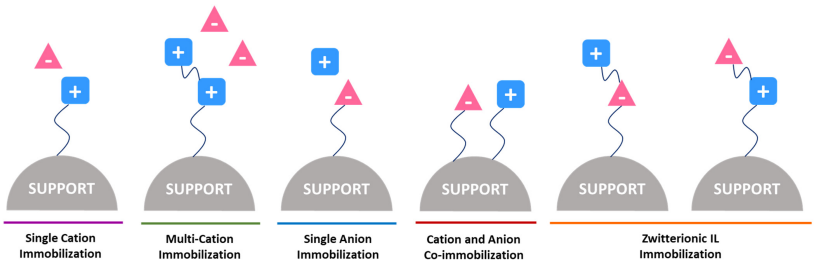

Figure 1. Illustration of distinct methods of IL immobilization onto a chromatographic support. From left to right: In the purple line is the cation immobilized on the support while the anion is free; in the green line is represented a multi-cation immobilization, with multi-anion as counter-ions; in the blue line is the anion immobilized on the support, while the cation is free; in the red line is represented the cation and the anion co-immobilized, where both are covalently bound to the matrix; In the orange line is represented zwitterionic IL immobilization, where the cation and anion are linked through a covalent bond.

In **Figure 1**, represented by the purple line, is the case where the IL cation is covalently attached to the solid support while the anion acts as a free counterion. This case is relatively easy to prepare and has the advantage that free anions can be easily replaced, making a simple regeneration processes, or even exchanged, enabling slight modifications by changing the anion [16]. Spherical porous silica is often used as a stationary phase matrix for HPLC, in which an imidazolium ring (the most used IL for covalent modification) is immobilized with a spacer arm. This type of stationary phase has already proven to be efficient in the separation of alkaloids, inorganic anions and cations [35], xylose, and glucose [36]. Additionally, the imidazolium-based support can promote different types of interactions, demonstrating hydrophobic and ionic properties, and, hence, enabling multi-modal separations. Usually, the cation is anchored on the silica by a small spacer arm, and different lengths of these spacers may influence the selectivity of the support by alteration of hydrophilicity [37][26][38]. Moreover, in a research performed by Neves et al. [39], where a macroporous support was functionalized with 1-methyl-3-propylimidazolium chloride, the establishment of different types of interactions between the ILs and biomolecules, such as gDNA and RNA, was also proven, enabling the effective separation of these two species. Several aspects point to the potential of ILs as truly multi-modal ligands, considering their ability to interact with analytes through different mechanisms, including hydrophobic, electrostatic, hydrogen bonding, π–π, and dipole–dipole interactions, due to their unique structure that comprises hydrophobic, hydrophilic and ionic moieties.

The green line in **Figure 1** also represents an IL immobilization with multi-cation moieties. In a research from Qiao et al. [39], it was shown that stationary phases with immobilized dicationic ILs presented effective retention and good selectivity for typical hydrophilic compounds under the HILIC mode, as well as an improved column efficiency. On the other hand, anions are rarely immobilized onto the supports (**Figure 1**, blue line), since a particular research of Qiu et al. [40] demonstrated that, in this case, the counterions are easily exchanged by the ionic species present on the mobile phases during usage. This effect greatly compromises the stability of the IL-based support and restrains the reproducibility of separations due to the possible exchange of the counterions affecting the interactions established.

The red line in **Figure 1** shows the co-immobilization of the cation and the anion onto the solid support. This strategy can improve the stability of these ligands during the use of different types of buffers in the mobile phase, thus influencing the selectivity by the distribution of polar groups [25][41]. Finally, in the orange line of **Figure 1** are given the examples of zwitterionic compounds, which can avoid the previous issues, once the cation and anion are covalently bound to each other, varying between which one is immobilized onto the solid support. Qiao et al. [42] developed a zwitterionic stationary phase with a positively charged imidazole ring and a negatively charged sulfonate group, which exhibited good selectivity and favorable retention for a wide range of polar solutes, including nucleosides and nucleic acids.

Thus, SILs allow a wide variety of interactions since they present both a positive and negative charge, which enables many electrostatic interactions according to the buffer pH and the biomolecule of interest. Furthermore, they also present functional groups, such as an imidazole ring, amine, and carboxyl groups that promote several types of interactions (hydrogen bonds and van der Waals, among others). They even can have alkyl chains (with various lengths) that strongly favor hydrophobic interactions with the different molecules of interest. All of this justifies ILs' applicability as promising ligands in chromatography once they can act as multimodal ligands [14][21].

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