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Synthesis and Characterization of Fluorinated Phosphonium Ionic Liquids to Use as New Engineering Solvents

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Abstract: In this work, a set of novel fluorinated ionic liquids (FILs), based on different tetraalkyl-phosphonium cations with perfluorobutanesulfonate and perfluoropentanoate anions, were synthesized and characterized in order to check their suitability to apply as engineering solvents. Thermophysical and thermal properties were both determined between 293.15 and 353.15 K, and the molecular volumes and free volumes and the coefficients of isobaric thermal expansion were determined from experimental values of refractive index and density. Lastly, the Walden plot was used to evaluate the ionicity of the novel FILs. The cytotoxicity of these compounds was also determined using the human hepatocellular carcinoma cells (HepG2) and the human colon carcinoma cells (Caco-2). Finally, the results were all discussed with the aim of understanding the behaviour of these compounds, considering the influence of the anion and the hydrogenated alkyl chain length. In summary, the new FILs synthesized in this work present adequate properties for their application in different industrial processes. Most of these compounds are liquid at room temperature with high decomposition temperatures. All phosphonium-based FILs have lower densities than conventional ionic liquids and common organic solvents, and the viscosity depends directly on the selected anion. Furthermore, the ionicity of FILs based on the sulfonate anion is higher than those based on the carboxylate anion. Finally, the phosphonium-based FILs have no significant effect on cellular viability at lower concentrations.

Keywords: synthesis; fluorinated ionic liquids; thermal properties; thermophysical properties; cytotoxicity

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1. Introduction

Ionic liquids (ILs) are novel salts with melting points close to or lower than 100 °C, which is a much lower temperature than that of traditional inorganic salts. The first generation of ILs is constituted by cations derived from alkylpyridinium and dialkylimidazolium conjugated with chloroaluminate and metal-halides-based anions [1]. This first generation appeared in the 1960s and was characterized by its air and water sensitivity. In the 1990s, the second generation of ILs, based on PF_6 and BF_4 anions, showed better stability than the first generation and was applied in various fields of chemistry [1–3]. Finally, the third generation of ILs was characterized by their biodegradability and improved ionic availability, and together with the deep eutectic solvents, third-generation ILs have become a hot topic in the scientific community [1–3].

These compounds have high ionic conductivity, low volatility and electrochemical stability, high thermal stability, and tuneable solubility [4,5]. Furthermore, the amount and versatility of ILs, which can be specifically designed taking into account the different combinations of the anions and cations, allow the proper selection of ILs for each specific

application [6]. These compounds are used in different fields, such as in analytical chemistry, separation and extraction processes, catalysis, and chromatography [7]. Biologically, they have also been used in drug delivery systems and as alternative solvents [8]. In the field of engineering, they have been applied as hydraulic fluids in liquid ring pumps and as hydraulic oil in diaphragm pumps, as well as lubricants for combustion engines, due to their high stability against oxidation [9].

Specifically, phosphonium-based ILs have high stability in basic media due to the lack of acidic proton production, and compared to ammonium salts, they have better thermal stability [10,11]. This unique family of ILs is being applied in many areas, such as for the extraction of noble metals in aqueous solutions [12], as well as supercapacitors due to their high performance at room temperature [13]. These ILs have also been tested as battery electrolytes, obtaining promising results [14], and they have shown anticorrosive properties when functionalized with sepiolite for epoxy resin coatings [15]. Additionally, they have been found that the different cations and anions and the variation of substituents in the cation change its anti-tumour activity against NCI 60 human tumour cell lines, favouring the control of its therapeutic properties [16]. The aggregation and chain length of both phosphonium-based IL cation and anion influence their toxicity in both Chinese hamster ovary cells and zebrafish (*Danio rerio*), where higher toxicity is associated with an increase in the alkyl chain length [17].

Fluorinated ionic liquids (FILs) are an unconventional family of ILs with fluorine atoms in their anion and/or cation. FILs combine the best properties possessed by perfluorinated compounds and conventional ILs, such as low surface tension, capacity to dissolve larger amounts of compounds, chemical and biological inertness, negligible vapour pressure, high surfactant power, wide liquid range, and easy recovery and recyclability, among others [18]. FILs must have fluorinated alkyl chains length equal to or bigger than 4 carbon atoms and exhibit different behaviour in many aspects, such as their high hydrophobicity and polarity compared to conventional ILs [19]. Then, FILs can also form three different nanodomains (polar, apolar, and fluorinated), which allow the accommodation and solubilization of different types of solutes [20]. Regarding the aggregation behaviour, FILs with bigger alkyl chain lengths can form different aggregates with more packed structures at lower concentrations than traditional surfactants [20,21]. Furthermore, FILs can be designed to improve their aggregation properties, enhancing their thermal stability and biocompatibility [20]. Regarding their cytotoxicity and ecotoxicity, it has been found that short alkyl-chain-based FILs conjugated with perfluorobutanesulfonate and perfluoropentanoate anions, $[C_4F_9SO_3]^-$ and $[C_4F_9CO_2]^-$, respectively, are not considered toxic [20,22,23].

FILs have been studied for biomedical applications, such as in drug delivery systems. They can be an effective solution to the delivery of therapeutic proteins because FILs can encapsulate these biomolecules and do not change their activity, stability, and secondary structure [20]. The numerous combinations of cation and anions allow the design of very attractive FILs for biomedical applications. This design can make them exceptional candidates for use as stabilizers, additives, or support as nano-encapsulation platforms that preserve the stability and activity of biomolecules, allowing their application as therapeutic biopharmaceuticals or devices [20]. In the separation processes of greenhouse gases, it has also been demonstrated that FILs can be used to reduce substantially harmful emissions by pollutants such as fluorinated gases: 1,1,1,2-tetrafluoroethane (R-134a), pentafluoroethane (R-125), and difluoromethane (R-32) [20].

Clearly, the chemical and pharmaceutical industry needs new solvents that improve industrial processes, minimizing their costs and their environmental and societal impacts [24–26]. With this objective in mind, the synthesis and characterization of new engineering solvents are fundamental. Therefore, the main purpose of this work is to synthesize and characterize six FILs based on tetra-alkyl-phosphonium cation with perfluorobutanesulfonate or perfluoropentanoate anions with the aim of evaluating if these FILs are suitable to be used as novel engineering solvents. The respective characterization was carried out on each FIL where thermal properties (glass transitions (T_g), solid-solid phase

transitions ($T_{s\text{-}s}$), start temperature (T_{start}), decomposition temperature (T_{dec}), onset temperature (T_{onset})), and physical properties (viscosity, density, refractive index, coefficients of isobaric thermal expansion, molecular and free volumes, conductivity, and ionicity) were determined. Furthermore, the cytotoxicity of these FILs was also determined using the human hepatocellular carcinoma cells (HepG2) and the human colon carcinoma cells (Caco-2). For thermal, thermophysical, and cytotoxicity properties, the results were analyzed and discussed according to the length of the alkyl hydrogenated chain and anion type.

2. Materials and Methods

2.1. Materials

Trihexyltetradecylphosphonium chloride, $[P_{66614}]Cl$ (>95% mass fraction purity); tributyltetradecylphosphonium chloride, $[P_{44414}]Cl$ (>95% mass fraction purity); and tetrabutylphosphonium bromide, $[P_{4444}]Br$ (>95% mass fraction purity), were provided by IoLiTec (Heilbronn, Germany). Perfluorobutanesulfonate acid, $C_4F_9SO_3H$ (>98% mass fraction purity), was provided by Tokyo Chemical Industry (Zwijndrecht, Belgium), and perfluoropentanoate acid, $C_4F_9CO_2H$ (>97% mass fraction purity), was provided by Fluorochem (Derbyshire, United Kingdom). These compounds were used to synthesize the FILs studied in this work. The name, acronym, and structures of the synthesized FILs are presented in Table 1. The human colon carcinoma cells (Caco-2) were supplied by Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Leibniz Institute, Braunschweig, Germany), and human hepatocellular carcinoma cells (HepG2) were provided by the European Collection of Cell Culture (ECACC, United Kingdom). MEM and RPMI 1640 medium and the corresponding supplements (L-Glutamine, fetal bovine serum (FBS), penicillin-streptomycin solution, sodium pyruvate, MEM nonessential amino acids (MEM-NEAA), and trypsin-EDTA solution) were supplied by Gibco (Invitrogen Corporation, Massachusetts, United States). Finally, a CellTiter 96® Aqueous One Solution Cell Proliferation Assay from Promega (Madison, Wisconsin, United States) was used to determine the cell viability.

Table 1. Structures of the synthesized fluorinated ionic liquids.

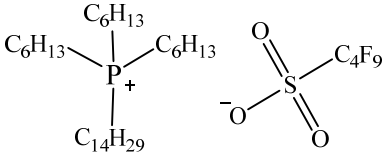
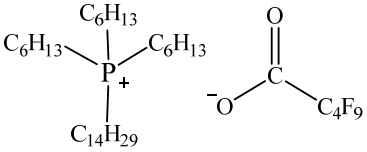
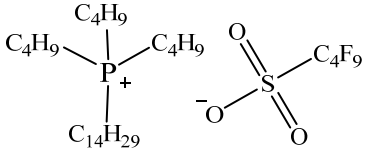
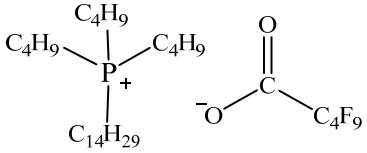
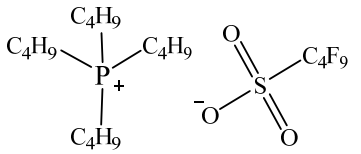
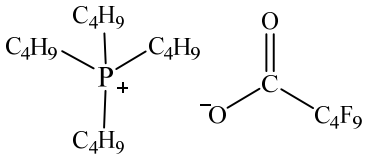
Name and Acronym	Chemical Structure
Trihexyltetradecylphosphonium perfluorobutanesulfonate $[P_{66614}][C_4F_9SO_3]$	
Trihexyltetradecylphosphonium perfluoropentanoate $[P_{66614}][C_4F_9CO_2]$	
Tributyltetradecylphosphonium perfluorobutanesulfonate $[P_{44414}][C_4F_9SO_3]$	
Tributyltetradecylphosphonium perfluoropentanoate $[P_{44414}][C_4F_9CO_2]$	

Table 1. *Cont.*

Name and Acronym	Chemical Structure
Tetrabutylphosphonium perfluorobutanesulfonate $[P_{4444}][C_4F_9SO_3]$	
Tetrabutylphosphonium perfluoropentanoate $[P_{4444}][C_4F_9CO_2]$	

The ion exchange resin method developed by Ohno et al. [27] was used to synthesize the FILs [21–23]. In this procedure, an anion exchange resin (Alfa Aesar Amberlite IRN78, Thermo Fisher Scientific, Waltham, United States) column was used to prepare different hydroxide solutions of phosphonium from phosphonium chlorides or bromides. Phosphonium hydroxides were neutralized by the dropwise incorporation of different fluorinated acid solutions. The synthesis procedures of each FILs are detailed in the Supplementary Materials (SM).

The purity of synthesized FILs was verified by ^{1}H, ^{19}F NMR, and elemental analysis, and their characterization is detailed in the SM. The integration of the ^{1}H and ^{19}F NMR peaks using an internal standard (dimethylsulfoxide, supplied by Eurisotop®, CEA Saclay, Gif sur Yvette, France) reveal the expected cation/anion correlation. All synthesized FILs were dried (under vacuum at 4×10^{-3} kPa and 303.15 K) to guarantee the absence of water and volatile substances contents before use.

2.2. Experimental Procedure

2.2.1. Thermal Properties

Thermogravimetric analysis (TGA) was performed with thermogravimetric analysis equipment (LABSYS Evo TGA from SETARAM) to study the weight variations of FILs with the increment of the temperature and, consequently, their decomposition temperature. Dry nitrogen gas was employed for the TGA experiments using a flow rate of 50 mL·min^{-1}. A total of 20–60 mg of FIL was incorporated into aluminium pans, and the temperature was increased until complete thermal degradation (maximum of 773 K at a rate of 1 K·min^{-1}) with an uncertainty of ±1 K in the temperature. Analysis was performed to obtain the starting (T_{start}), onset (T_{onset}), and decomposition (T_{dec}) temperatures which are related to the temperature at which the weight loss was 1% in the determination of T_{start}, the baseline slope changed during the run in the determination of T_{onset}, and the weight loss was 50% in the determination of T_{dec}. These temperatures were calculated using the Calisto processing software.

Glass transitions (T_g), solid-solid transitions ($T_{s\text{-}s}$), and melting points (T_m) of the synthesized FIL were studied in a Q2000 differential scanning calorimeter (DSC of the TA instrument) with a chilled cooling system. Approximately 5–8 mg of FIL was placed in a sealed aluminium pan, and the sample was continuously purged with dry nitrogen at a flow rate of 50 cm^3·min^{-1}. The cooling–heating runs were carried out at 1 K·min^{-1}, 5 K·min^{-1}, and10 K·min^{-1}. The best rate was selected for the synthesized FIL in order to determine the various phase transitions in a more precise way. An uncertainty of ±1 K was determined. The different phase transitions were determined using the Universal Analysis 2000 v4.5A software of the TA Instrument.

2.2.2. Viscosity and Density

FILs' dynamic viscosity and density at atmospheric pressure and between 293.15 and 353.15 K were measured in an SVM 3000 Anton Paar rotational Stabinger viscometer–densimeter. The calibration of the instrument was checked with bi-distilled water. These measurements have a temperature uncertainty of 0.02 K. Regarding the dynamic viscosity, two independent samples were measured, and the reported result is the average value with a relative standard deviation (RSD) of 1% and overall uncertainty of 2% (taking into account the handling of the samples and purity). On the other hand, duplicates of density measurements were carried out, and an uncertainty of 0.0002 $g \cdot cm^{-3}$ was determined.

2.2.3. Ionic Conductivity

Ionic conductivities were determined using an FP30 conductimeter (Mettler Toledo Group) within a glass jacketed cell which contains a magnetic agitator. The cell was thermostabilized with an A100 water bath (LAUDA with an uncertainty of ± 0.01 K). A platinum resistance thermometer connected to a Keithley 199 System DMM/Scanner was used to measure the temperature (with an uncertainty of ± 0.01 K). Approximately 1.5 mL of each FIL was incorporated into the thermostatic cell (closed to ensure a secure seal) and continuously stirred. The conductimeter was firstly calibrated in the temperature range studied with certified standard solutions of Radiometer Analytical (0.01 D and 0.1 D of KCl). Duplicates of conductivity measurements were carried out at atmospheric pressure and between 293.15 and 323.15 K. An uncertainty of $\pm 2\%$ was estimated for these experimental measurements. The ionic conductivity measurements were previously validated, obtaining a maximum deviation of 2% [28].

2.2.4. Refractive Index

The refractive index of the synthesized FILs was measured at atmospheric pressure and between 293.15 and 353.15 K with an ABBEMAT 500 Anton Paar automatic refractometer which has a resolution of $\pm 10^{-6}$. Every refractive index was determined at least two times with an uncertainty of $\pm 5 \times 10^{-5}$. The refractometer was calibrated with tetrachloroethylene and verified before the measurements with Millipore quality water.

2.2.5. Cytotoxicity Assays

Two different human cell lines (human colon carcinoma cells, Caco-2, and human hepatocellular carcinoma cells, HepG2) were chosen because they represent different pharmaceutical administration routes. Cell culture routine and cytotoxicity assays were performed accordingly to the protocol already implemented in our lab [20,23]. FILs toxicity towards Caco-2 and HepG2 was tested in a concentration range between 1 and 10,000 μmL^{-1}. In the culture medium, these FILs present poor solubility. Then, FIL solution was firstly prepared in DMSO and after was diluted in 0.5% FBS culture medium (1% v/v DMSO maximum value). Cellular viability was measured after 24 h incubation with FILs solutions. DMSO (1% v/v as a maximum value) were prepared in a culture medium for the negative controls, and solutions containing only DMSO (100% v/v as a maximum value) were used for positive controls. The samples were incubated for 24 h, and after they were removed. Then, 100 µL of CellTiter 96® Aqueous One Solution Cell Proliferation Assay reagent was incorporated into the different wells and incubated for 4 h. After incubation, a Thermo Scientific Multiskan GO microplate reader (Waltham, MA, USA) was used to measure the absorbance at 490 nm. Triplicates of each FIL concentration and controls were measured in three independent assays. Cellular viability was calculated by the ratio made between the absorbance of the cells in contact with FIL-based solutions and the absorbance of the negative control cells. The cellular viability trends were used to determine the dose-dependent viability curves. Additionally, the effective concentration (EC_{50} value), which is the FIL concentration required to reduce cell viability by 50%, was determined using these dose-response curves.

3. Results and Discussion

3.1. Thermal Properties

FILs can be designed as green solvents and become promising alternatives for several industrial and biomedical processes. However, before any application, it is essential to ascertain the working temperature range for each one of the studied FILs. Melting temperature allows us to know the temperature at which the compounds become liquid, while the decomposition temperature shows us the maximum working temperature before compound degradation [18,21]. FILs were thermally characterized, and all phase transitions and decomposition temperatures were identified in Tables 2 and 3 and illustrated in Figures S1–S6 of SM. All compounds are liquid at room temperature except for the FILs based on $[P_{4444}]^+$ cation, which remain solid in all ranges of studied temperatures, which may be related to the high symmetry of this phosphonium cation.

Table 2. Glass transitions (T_g) and solid-solid transitions (T_{s-s}) of the fluorinated ionic liquids.

Ionic Liquid	T_g [K]	T_{s-s} [K]
$[P_{66614}][C_4F_9SO_3]$	201	-
$[P_{66614}][C_4F_9CO_2]$	196	-
$[P_{44414}][C_4F_9SO_3]$	211	-
$[P_{44414}][C_4F_9CO_2]$	205	-
$[P_{4444}][C_4F_9SO_3]$	221	247
$[P_{4444}][C_4F_9CO_2]$	-	253

Table 3. Degradation properties: start temperature—T_{start}; onset temperature—T_{onset}; decomposition temperature—T_{dec}, of the studied fluorinated ionic liquids.

Ionic Liquid	T_{start} 1[K]	T_{onset} 1[K]	T_{dec} 1[K]
$[P_{66614}][C_4F_9SO_3]$	499	673	670
$[P_{66614}][C_4F_9CO_2]$	378	421	413
$[P_{44414}][C_4F_9SO_3]$	484	669	676
$[P_{44414}][C_4F_9CO_2]$	374	420	413
$[P_{4444}][C_4F_9SO_3]$	492	669	664
$[P_{4444}][C_4F_9CO_2]$	494	669	665
	T_{start} 2[K]	T_{onset} 2[K]	T_{dec} 2[K]
$[P_{66614}][C_4F_9CO_2]$	435	529	514
$[P_{44414}][C_4F_9CO_2]$	440	473	499
	T_{start} 3[K]	T_{onset} 3[K]	T_{dec} 3[K]
$[P_{66614}][C_4F_9CO_2]$	552	608	609
$[P_{44414}][C_4F_9CO_2]$	586	704	691

The results obtained suggest that FILs with the shorter hydrogenated alkyl chain length have higher T_g than the ones based on the long alkyl chain length, such as the $[P_{66614}]^+$ cation. Furthermore, sulfonate-based FILs also have a higher T_g than carboxylate-based ones. This temperature indicates the change from a rigid, solid amorphous crystal to a more flexible amorphous crystal [29]; all FILs exhibit a glass transition temperature except $[P_{4444}][C_4F_9CO_2]$. It was also observed a T_{s-s} in both $[P_{4444}][C_4F_9SO_3]$ and $[P_{4444}][C_4F_9CO_2]$ FILs, which represents a transition between two different solid structures. Neither of the FILs based on $[P_{4444}]^+$ cation revealed a melting point in all the operation range of DSC equipment.

The degradation temperatures of all synthesized FILs are indicated in Table 3, based on the obtained thermograms represented in Figures S7–S12 of SM. The analysis of the thermograms suggests that the degradation of the FILs based on the carboxylate anion conjugated with longer alkyl chain length-based cations ($[P_{66614}]^+$ and ($[P_{44414}]^+$) occurs at different stages. This behaviour when IL degrades in different stages is also observable in literature works [30,31]. For $[P_{66614}][C_4F_9CO_2]$ and $[P_{44414}][C_4F_9CO_2]$, these stepwise degradations can be a result of the different structural arrangement between the anion and

cation in comparison to the sulfonate-based ones [21]. This degradation at different stages is identified in Table 3 with the numbers 1–3.

These results indicate that the increment of the hydrogen chain has a very small effect on the decomposition temperatures of these compounds. Moreover, FILs with the carboxylate anion have lower decomposition temperatures than those based on the sulfonate anion, except for FILs based on $[P_{4444}]^+$ cation, where very similar decomposition temperatures are obtained. $[P_{44414}][C_4F_9SO_3]$ is the FIL with the highest decomposition temperature, and it is considered the most stable compound at higher operating temperatures.

3.2. Thermophysical Properties

FILs thermophysical properties are indispensable for their application as engineering solvents in the development and upgrade of industrial, chemical, and pharmaceutical processes. Density is an essential and relevant characteristic for fluid transportation and different processes such as liquid-liquid extraction and other separation processes [18]. In this work, the relationship between temperature and density was analyzed through the following expression widely used in literature to correlate these experimental data [18,21]:

$$\ln \rho = A_0 + A_1 T \tag{1}$$

where A_0 and A_1 are adjustable parameters and T is the temperature. Density data were correlated using a linear fitting with reduced deviations (S.D.) for density experimental values. Density values for the studied FILs are depicted in Figure 1 and reported in Table S1 of the SM, and the correlation parameters and deviations have also been listened to in Table S2 of the SM. The errors between experimental and calculated data were determined through the calculation of the standard deviations, which were obtained as follows:

$$\text{S.D.} = \left(\frac{\sum_i^{n_{DAT}} \left(Z_{exp} - Z_{adjust} \right)^2}{n_{dat}} \right)^{\frac{1}{2}} \tag{2}$$

where n_{DAT} represents the amount of experimental and adjustable data, and Z represents the property values.

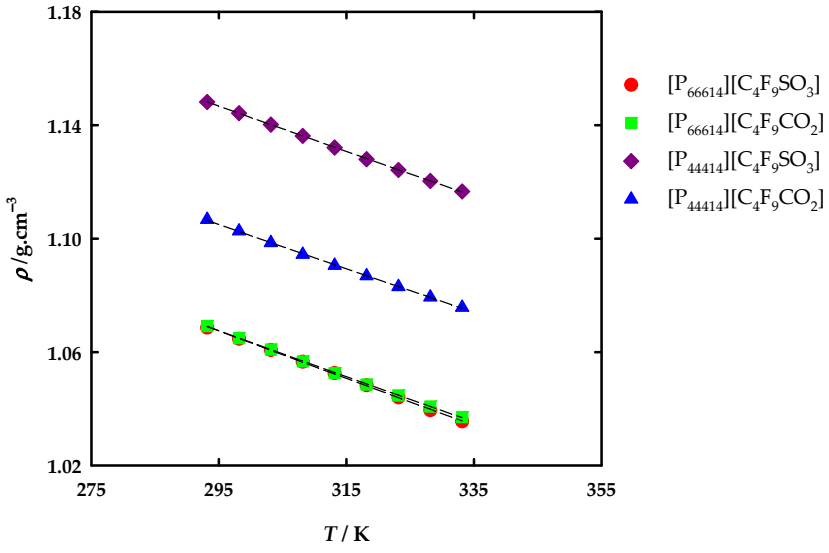


Figure 1. Density values as a function of temperature (T) and fitted curves for the synthesized fluorinated ionic liquids.

The analysis of the results shows that the increase in the hydrogenated alkyl chain length leads to a reduction of density in all ranges of tested temperatures, as follows:

$[P_{44414}][C_4F_9SO_3] > [P_{44414}][C_4F_9CO_2] > [P_{66614}][C_4F_9SO_3] \approx [P_{66614}][C_4F_9CO_2]$. Furthermore, FILs with the sulfonate anion have a higher density than those based on the carboxylate anion, except for FILs with $[P_{66614}]^+$ cation, where no significant differences were observed. This behaviour was also observed in work developed by Vieira et al. [21], where imidazolium ILs based on the same anions show that the increase in the hydrogenated alkyl chain length is associated with a reduction of the density values, and carboxylate anions showed lower density values than FILs based on sulfonate anion with identical cation. The validation of the density results obtained in this work was carried out with the available literature values for $[P_{66614}][C_4F_9SO_3]$ and $[P_{66614}][C_4F_9CO_2]$ FILs [32–34], obtaining a maximum deviation of 2%, which corroborates the quality of the results obtained in this work. The differences obtained between experimental and literature data are acceptable and associated with the different experimental techniques applied in both studies and from distinct FILs sources and handling procedures [35,36].

The isobaric thermal expansion coefficient, α_p, is calculated as the temperature derivative of $\ln(\rho)$. Then, this coefficient for these FILs corresponds to parameter A_1 of Equation (1) [21], as shown in equation:

$$\alpha_p\left(K^{-1}\right) = -[\partial \ln \rho / \partial T(K)]_p \quad (3)$$

The results of these coefficients for the studied FILs are illustrated in Figure 2. Based on the definition of α_p, the use of a linear fitting originates a thermal expansion coefficient independent of the temperature. The analysis of the results shows that the increase in the hydrogenated alkyl chain length induces an increment in their isobaric thermal expansion coefficients. Additionally, it is not possible to extract a behaviour in anion effect on this property. The imidazolium-based FILs [21] demonstrated the same behaviour as the phosphonium FILs where α_p increases with the increment in the cation of the alkyl chain length.

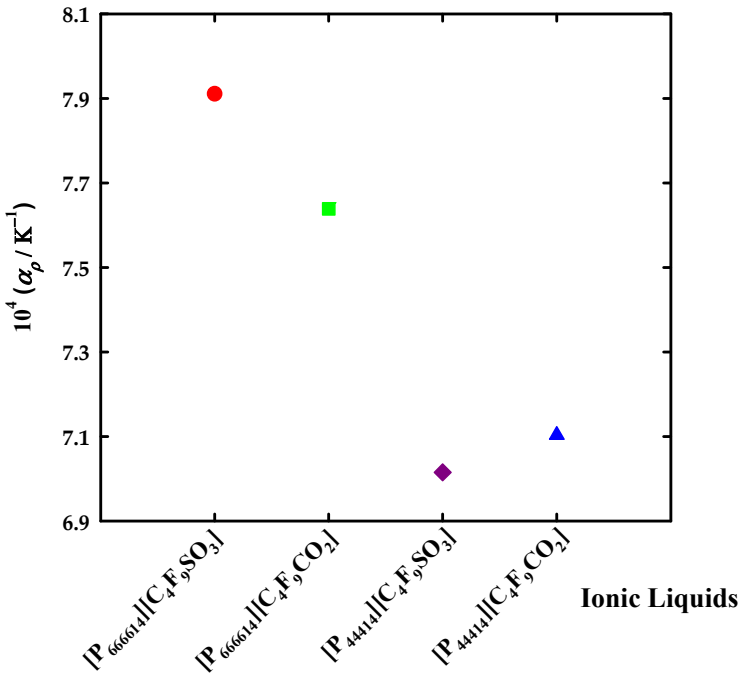


Figure 2. Isobaric thermal expansion coefficients (α_p) for the synthesized fluorinated ionic liquids.

On the other hand, the refractive index is considered a measurement of the electronic polarizability of a compound, providing knowledge about the forces between molecules [37] and their behaviour when they are in solution [38,39]. The refractive index, n_D, can be

related to the electronic polarizability, α_e, using Lorenz–Lorentz equation, which can be defined using the molar polarizability [40], R_m, or the molar refraction as follows:

$$R_m = \frac{N_A \alpha_e}{3\varepsilon_0} = \left(\frac{n_D^2 - 1}{n_D^2 + 2}\right) V_m \quad (4)$$

where ε is the dielectric constant, N_A is the Avogadro's constant, and V_m is the molar volume. R_m and V_m of the synthesized FILs were determined and detailed in Table S3 of the SM. Unsurprisingly, both molar refractions and molar volumes increase with longer hydrogenated alkyl chain lengths. Molar refractions and molar volumes for FILs with the sulfonate anion present slightly greater values than FILs with the carboxylate anion: $[P_{44414}][C_4F_9SO_3] > [P_{44414}][C_4F_9CO_2]$ and $[P_{66614}][C_4F_9SO_3] > [P_{66614}][C_4F_9CO_2]$.

Viscosity is a transport property essential to any fluid phase and evaluates the resistance exerted by a compound that is being deformed by tensile or shear stress [40]. Viscosity also allows understanding of the tested compound and facilitates mass transfer in any process that involves mass transfer operations such as pumping, mixing, or agitation because high viscosity values lead to difficulties in these operations [40].

Viscosity is temperature-dependent. Therefore, experiments were carried out between 293.15 and 353.15 K. These experimental results are plotted in Figure 3 and shown in Table S1 of the SM. Arrhenius fittings for dynamic viscosity, η, were well represented with the Vogel–Fulcher–Tammann (VFT) equation widely used in the literature to correlate the viscosity data of ILs [21,28]:

$$\ln \eta = \ln \eta_0 + \frac{B}{T - T_0} \quad (5)$$

where η_0, B, and T_0 are constants. These constants are shown in Table S2 of the SM together with the corresponding standard deviations (S.D.), which were determined with Equation (2). Viscosity data plotted in Figure 3 reveal that the increase in the hydrogenated alkyl chain length reduces the FILs' viscosity. Regarding the anion effect, the sulfonate-based FILs show higher dynamic viscosity than carboxylate-based FILs. The same behaviour was observed in imidazolium-based FILs [21], where the increase in the hydrogenated alkyl chain length is connected to a reduction of viscosity values as well as the sulfonate-based FILs reveal higher viscosity than carboxylate-based. The FIL with higher viscosity values ($[P_{44414}][C_4F_9SO_3]$) could lead to difficulties in mass transfer operations. The validation of our experimental data was carried out with those existing in literature [32,33], and a maximum deviation of $\pm$14% and $\pm$17% is achieved for $[P_{66614}][C_4F_9SO_3]$ and $[P_{66614}][C_4F_9SO_3]$, respectively. These deviations are a result of the distinct purities, water content, and handling of the samples, as well as the different measuring methods.

On the other hand, a comparison with other ILs [18,41,42] and a conventional solvent (sulfonale) commonly used in aromatic/aliphatic separations [43,44] was carried out in Figure 4. The traditional ILs selected to compare to the novel synthesized FILs based on $[P_{44414}]^+$ cation are based on two different cations (1-butyl-3-methylimidazolium, $[C_4C_1Im]^+$, and tetrabutylammonium, $[N_{4444}]^+$) and three different anions (hexafluorophosphate, $[PF_6]^-$, trifluoromethanesulfonate, $[CF_3SO_3]^-$, and perfluorobutanesulfonate, $[C_4F_9SO_3]^-$). The analysis of the values of the densities shows that the neoteric phosphonium-based FILs have lower density values than sulfolane and the other traditional ILs selected in this comparison. Clearly, bulky cations, such as $[P_{44414}]^+$ and $[N_{4444}]^+$, decrease density values, as expected, obtaining lower density values than common organic solvents such as sulfolane. However, this behaviour is not observed in the values of dynamic viscosity, where the influence of the anion clearly has a clear effect on the values of this transport property. The highest viscosity values are obtained for ILs based on bulky anions such as the $[C_4F_9SO_3]^-$ anion, followed by ILs based on the $[PF_6]^-$ and $[C_4F_9CO_2]^-$ anions (which have similar values). The lowest values for this transport property are obtained for sulfolane followed by the IL based on the $[CF_3SO_3]^-$ anion (which is the smallest anion).

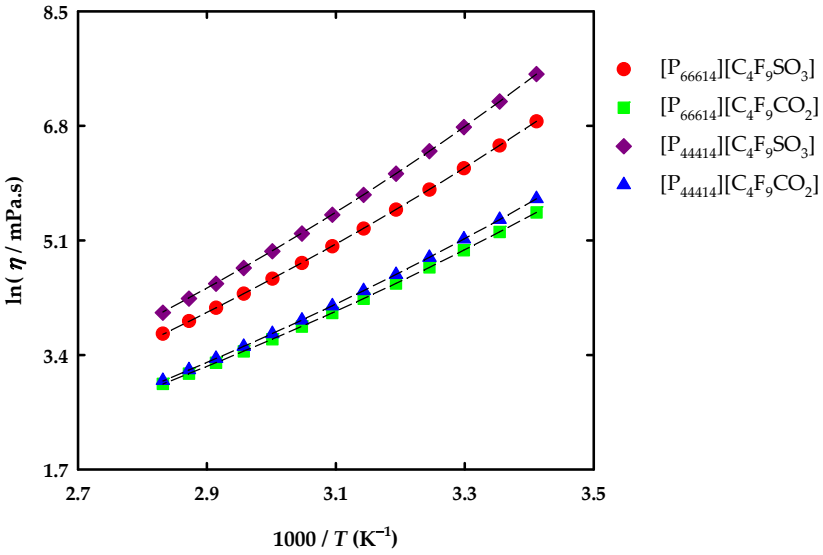


Figure 3. Arrhenius plots of the dynamic viscosity and the corresponding fitted curves for the synthesized fluorinated ionic liquids.

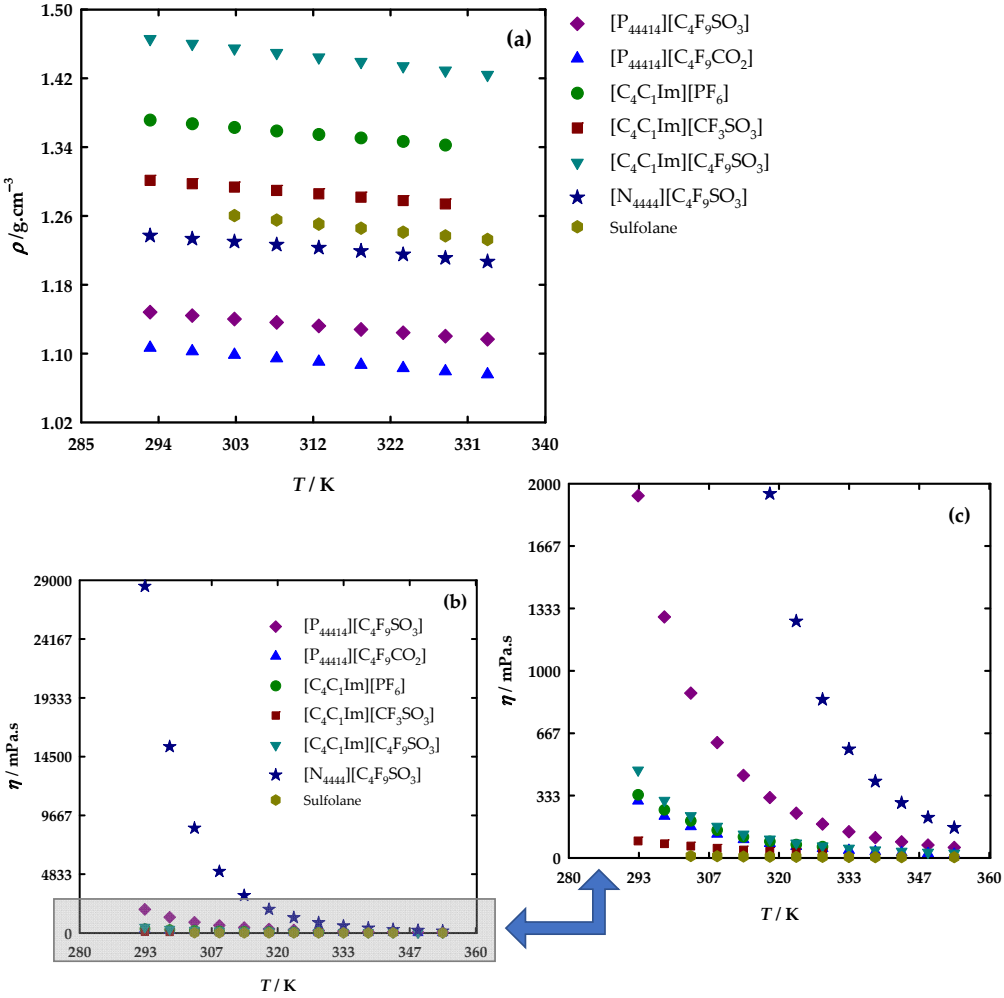


Figure 4. Comparison of: (**a**) density values; (**b**) dynamic viscosity; and with (**c**) an extension of dynamic viscosity for low values of the novel synthesized FILs based on $[P_{44414}]^+$ cation with traditional ILs [18,41,42] and a common organic solvent (sulfolane) [43].

Finally, ionicity is also a relevant property for industrial process purposes and provides relevant insights on the mobility of fluid and evaluates the possibility of the formation of aggregates [45]. Ionicity is calculated based on the molar electrical conductivity (obtained from the experimental conductivity) and the dynamic viscosity of the tested compound [18,21]. The experimental conductivity data are summarized in Table S1 of the SM and plotted in Figure 5. The temperature dependence of the experimental conductivity was also fitted as follows (VFT equation) previously used in the literature to correlate the ionic conductivity data of ILs [18,21,28]:

$$\ln k = \ln k_0 - \frac{B'}{T - T_0'} \quad (6)$$

where k_0, B', and $T_0{}'$ are constants and are summarized in Table S2 of the SM together with the best constants of the ionic conductivity.

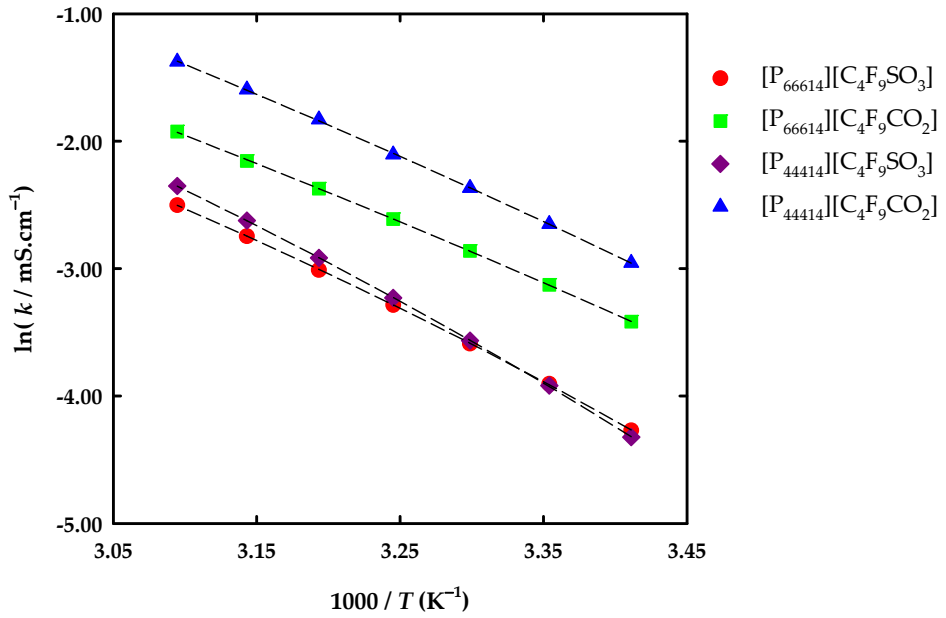


Figure 5. Arrhenius plots of experimental conductivity and the corresponding fitted curves for the synthesized fluorinated ionic liquids.

The analysis of the conductivity results shows that the increase in the hydrogenated alkyl chain length leads to a reduction of ionic conductivity values in all ranges of tested temperatures, except for FILs based on $[C_4F_9SO_3]^-$ anion, where no significant differences were observed. Furthermore, FILs with the sulfonate anion have lower conductivity values than FILs with the carboxylate anion.

The presence of aggregates and values of high viscosity in FILs can influence their mobility and molar conductivity, providing a behaviour away from the ideal electrolyte. One versatile mechanism to represent the ionicity of ionic liquids easily is the Walden plot since it relates the viscosity of a fluid with the molar conductivity [46–48]. Figure 6 represents the Walden plot of the studied FILs, in which the black straight line represents the behaviour of the "ideal" electrolyte (a solution of aqueous potassium chloride). This "ideal" electrolyte does have ions with equal mobility and is fully dissociated [49]. According to this graph, the FILs could be classified as either a "good" or a "poor" ionic fluids accordingly to their proximity to the behaviour of this black straight line, the ideal electrolyte [50].

Then, Figure 6, Walden plot, shows that the increase in the hydrogenated alkyl chain lengths decreases the ionicity of the FILs. Furthermore, FILs with the sulfonate anion have higher ionicity values than FILs with the carboxylate anion. Furthermore, it confirms a linear dependence with the temperature for all studied FIL, as expected. A comparison

with the imidazolium FILs [21] only reveals a similar behaviour to the phosphonium FILs: FILs with the sulfonate anion are closer to the ideal electrolyte in contrast with FILs with carboxylate anions.

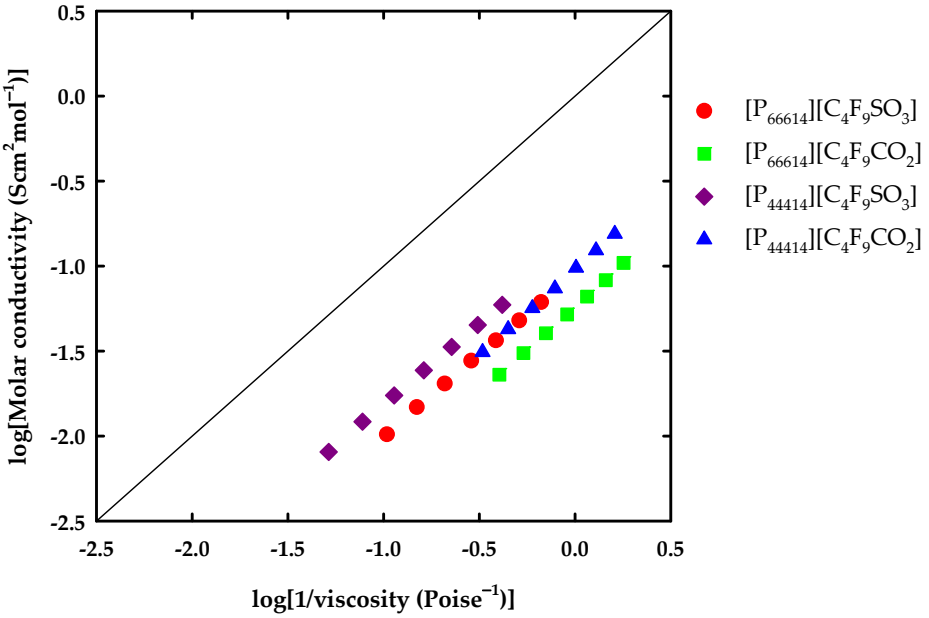


Figure 6. Walden plot for the synthesized fluorinated ionic liquids.

3.3. In Vitro Cell Viability Assays

The cytotoxicity assays are efficient tools for the selection of the most biocompatible FIL-based compounds before their application in industrial and biomedical areas. These assays are a cost-effective and time-saving method [18]. In this study, two human cell lines were selected to evaluate the cytotoxicity of the synthesized FILs. Human colon carcinoma cells (Caco-2) are commonly applied for toxicity assays and act as a model of intestinal physiology to evaluate the interaction and effect of the drugs, food, and chemical components [23,51–55]. Human hepatocellular carcinoma cells (HepG2) are also used in toxicity studies since they maintain the morphology and liver function for extended cycles and have higher metabolic activity than Caco-2 cells [18,23,56,57]. Additionally, these cell lines, Caco-2 and HepG2, can provide useful information regarding the toxicity of FILs-based systems for biomedical devices and drug delivery systems regarding an oral and rectal administration route [23].

The dose-response profiles for all FILs are represented in Figure 7, and the determined half-maximal effective dose (EC_{50}) values are detailed in Table 4. The effect of both anion type and cationic hydrogenated alkyl chain length in FILs cytotoxicity can be further discussed.

From the analysis of the obtained results, it can be demonstrated how FILs with longer hydrogenated chain lengths ($[P_{66614}]^+$) have a low influence on the viability of the Caco-2 cells, with a maximum reduction of 15% in cellular viability at the higher tested concentration (selected taking into account the solubility limit of these FILs in the cellular medium). This behaviour is similar to the study made with the $[P_{66614}][Ntf_2]$ (trihexyltetradecylphosphonium bis(trifluoromethylsulfonyl)imide) in the line cell Caco-2 by Frade et al. [58]. In the HepG2 cells, the reduction in the cellular viability is approximately 30% at the higher tested concentration. However, the concentrations tested in HepG2 are lower than those tested in Caco-2 because the solubility limits of these FILs in the cellular medium are lower. The same solubility limitation was observed with the carboxylate-based anion. However, it can be concluded that, at comparable concentrations, there is no relevant influence of the anion type in the cytotoxicity of these $[P_{66614}]^+$-based FILs. Although the EC_{50} is not achieved, we can conclude that all $[P_{66614}]^+$-based FILs are more toxic in the HepG2 cell

line compared to the Caco-2 cell line, obtaining a drop in cell viability at concentrations higher than 75 µM.

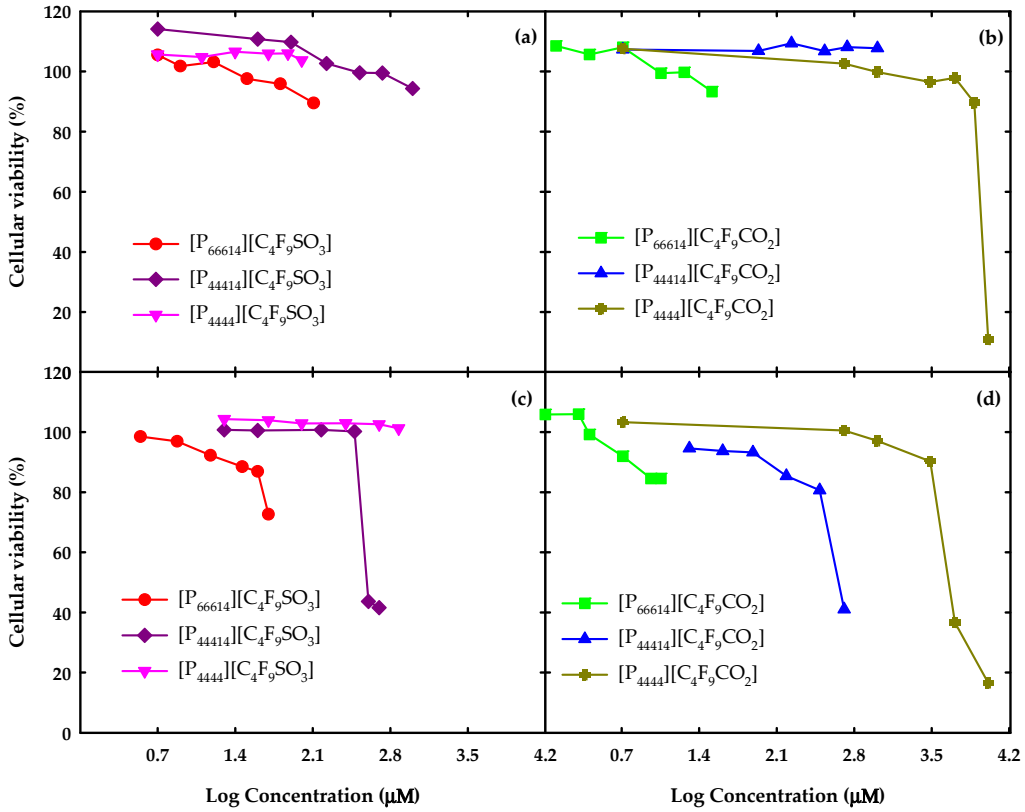


Figure 7. Cellular viability determined in this work for phosphonium synthesized FILs with: (**a**) the sulfonate anion in Caco-2 cell line; (**b**) the carboxylate anion in Caco-2 cell line; (**c**) the sulfonate anion in HepG2 cell line; and (**d**) the carboxylate anion in HepG2 cell line.

Table 4. Half-maximal effective dose (EC_{50}) for the Caco-2 and HepG2 cell lines after 24 h of FILs exposure.

	EC_{50} (µM)	
Ionic Liquid	**Caco-2**	**HepG2**
$[P_{66614}][C_4F_9SO_3]$	>128	>50
$[P_{66614}][C_4F_9CO_2]$	>32	>11
$[P_{44414}][C_4F_9SO_3]$	>1000	389
$[P_{44414}][C_4F_9CO_2]$	>1000	447
$[P_{4444}][C_4F_9SO_3]$	>100	>750
$[P_{4444}][C_4F_9CO_2]$	8710	7244

For $[P_{44414}]^+$-based FILs, it is possible to verify that, in the HepG2 cell line, there is a large drop in cell viability after a certain concentration (>75 µM) compared to the Caco-2 cell line. In the Caco-2 cell line, the $[P_{44414}]^+$-based FILs with the sulfonate anion have a drop in cell viability of 20%, while with the carboxylate anion, it is viable throughout the range studied. Some works show the higher toxicity of other $[P_{44414}]^+$-based ILs. For example, Mikkola et al. [59] studied $[P_{44414}][OAc]$ (tributyl(tetradecyl)phosphonium acetate) or $[P_{44414}]Cl$ (tributyl(tetradecyl)phosphonium chloride) in a human corneal epithelial cell line (HCE) or *E. coli* bacterial cells. In this work, they found that after a concentration of approximately 10µM of these ILs in HCE or *E. coli* bacterial cells, the viability is almost 0%.

FILs based on the $[P_{4444}]^+$ cation have similar cytotoxicity profiles in both cell lines. However, in this case, the sulfonate-based FIL is less soluble than carboxylate-based

compounds, and its solubility in the Caco-2 cells medium is lower than in the HepG2 medium. Nevertheless, at all tested concentrations, the cellular viability is maintained with the sulfonate-based FILs for both cell lines. Contrarily, the $[P_{4444}]^+$-based FILs conjugated with the carboxylate anions are soluble at very high concentrations in both cell lines. As depicted in Figure 7, the cytotoxic behaviour of $[P_{4444}][C_4F_9CO_2]$ is similar in both cell lines, being slightly more toxic to HepG2 cells with an EC_{50} value of 7080 μM than to Caco-2 cells with an EC_{50} value of 8710 μM (see Table 4). It is worth highlighting the reduced cytotoxicity of $[P_{4444}][C_4F_9CO_2]$, which was soluble in a broad range of tested concentrations and only had EC_{50} values at considerably higher concentrations. Comparing the $[P_{4444}]^+$-based FILs with some cytotoxicity data in the literature (tetrabutylphosphonium chloride, $[P_{4444}]Cl$, or tetrabutylphosphonium acetate, $[P_{4444}][OAc]$) with HCE or *E. coli* bacterial cells [59] and $[P_{4444}]Br$ (tetrabutylphosphonium bromide) in *Spodoptera frugiperda* 9 (Sf-9) cell line [60], we can conclude that in lower concentrations the $[P_{4444}]^+$-based ILs have not significant effect on cellular viability. Then, we can conclude that $[P_{4444}]^+$-based FILs can be used without biocompatibility issues at specific concentrations below their EC_{50} values or solubility limits.

4. Conclusions

In this work, the thermal and thermophysical properties (dynamic viscosity, density, refractive index, and ionic conductivity) were measured for fluorinated ionic liquids with three different alkyl side chains ($[P_{4444}]^+$, $[P_{44414}]^+$, and $[P_{66614}]^+$) and two different anions ($[C_4F_9SO_3]^-$ and $[C_4F_9CO_2]^-$). Moreover, the cytotoxicity in human hepatocellular carcinoma cells (HepG2) and human colon carcinoma cells (Caco-2) was also analyzed in order to evaluate their biocompatibility. A comparison between the different FILs was carried out, taking into account the anion effect and the increment of alkyl chain length. FILs with symmetric cations (($[P_{4444}]^+$) are the only ones that are solid at room temperature. However, most phosphonium-based FILs are liquids with high degradation temperatures. All these experimental data allow us to understand the behaviour and limits of these FILs to apply in different industrial operations and biomedical applications. The analysis of the results demonstrated a substantial dependency of these properties with the changes in the anion or the increment of the alkyl chain length. Moreover, FILs with the carboxylate anion show lower decomposition temperatures, density, viscosity, and ionicity but higher toxicity when compared with FILs with the sulfonate anion. The density of these new FILs is much lower than some traditional ionic liquids and common organic solvents, while the viscosity depends directly on the selected anion. Finally, these FILs can also be used without biocompatibility issues at definite concentrations below their EC_{50} values or solubility limits.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/chemengineering6030038/s1: Synthesis procedure, NMR, and elemental analysis; Figure S1: DSC curves at 1 K/min of $[P_{66614}][C_4F_9SO_3]$; Figure S2: DSC curves at 1 K/min of $[P_{66614}][C_4F_9CO_2]$; Figure S3: DSC curves at 10 K/min of $[P_{44414}][C_4F_9SO_3]$; Figure S4: DSC curves at 1 K/min of $[P_{44414}][C_4F_9CO_2]$; Figure S5: DSC curves at 1 K/min of $[P_{4444}][C_4F_9SO_3]$; Figure S6: DSC curves at 10 K/min of $[P_{4444}][C_4F_9CO_2]$; Figure S7: Thermogravimetric curves at 1 K/min of $[P_{66614}][C_4F_9SO_3]$; Figure S8: Thermogravimetric curves at 1 K/min of $[P_{66614}][C_4F_9CO_2]$; Figure S9: Thermogravimetric curves at 1 K/min of $[P_{44414}][C_4F_9SO_3]$; Figure S10: Thermogravimetric curves at 1 K/min of $[P_{44414}][C_4F_9CO_2]$; Figure S11: Thermogravimetric curves at 1 K/min of $[P_{4444}][C_4F_9SO_3]$; Figure S12: Thermogravimetric curves at 1 K/min of $[P_{4444}][C_4F_9CO_2]$; Table S1: Density, ρ, dynamic viscosity, η, refractive index, n_D, and ionic conductivity, k, of the fluorinated ionic liquids as a function of temperature; Table S2: Fitting parameters for the density, refractive index, viscosity and ionic conductivity as a function of temperature for fluorinated ionic liquids. Standard deviations (S.D.) are also shown; and Table S3: Values of calculated molar volume, V_m, and molar refraction, R_m, as a function of temperature for selected fluorinated ionic liquids.

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