



ОРИГИНАЛЬНЫЕ СТАТЬИ

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Sorption of flavonoids and aromatic acids on hypercrosslinked polystyrene in systems with addition of imidazolium ionic liquids

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Abstract. In this work the effect of the addition of five imidazolium ionic liquids (ILs) to the water-acetonitrile eluents on the sorption of aromatic acids and flavonoids on hypercrosslinked polystyrene (HPS) was evaluated by reversed-phase HPLC (RP HPLC). It was found that both the nature of the cation and the nature of the anion of the used IL influence the retention of the studied compounds. The retention of aromatic acids and flavonoids in the systems without and with the addition of trifluoroacetic acid (TFA) to the eluent was investigated. It was shown that the addition of TFA to the water-acetonitrile eluent changes the mechanism of action of imidazolium ILs on the retention of sorbates on HPS. In particular, the sequence of elution of ferulic acid, salicylic acid and caffeic acid in the systems with addition of ILs to the mobile phase on HPS varies depending on the pH of the medium (depend of the addition of TFA). The Snyder-Soczewiński and Soczewiński–Wachtmeister models were used to evaluate the influence of water-acetonitrile eluent composition on the retention of studied compounds. The angular coefficients of the corresponding models were calculated.

Keywords: imidazolium ionic liquids, hypercrosslinked polystyrene, reversed-phase high-performance liquid chromatography, flavonoids, aromatic acids

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Научная статья

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Сорбция флавоноидов и ароматических кислот на сверхсшитом полистироле в системах с добавками имидазолиевых ионных жидкостей

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Аннотация. В данной работе методом обращенно-фазовой ВЭЖХ (ОФ ВЭЖХ) оценено влияние добавок пяти имидазолиевых ионных жидкостей (ИЖ) к водно-ацетонитрильному элюенту на сорбцию ароматических кислот и флавоноидов на сверхсшитом полистироле (СПС). Было установлено, что на удержание исследуемых соединений влияют как природа катиона, так и природа аниона используемой



ИЖ. Изучено удерживание ароматических кислот и флавоноидов в системах без добавления и с добавлением в элюент трифторуксусной кислоты (ТФУ). Показано, что добавление ТФУ в водно-ацетонитрильный элюент изменяет механизм действия имидазолиевых ИЖ на удерживание сорбатов на СПС. В частности, порядок выхода феруловой, салициловой и кофейной кислот в системах с ИЖ на СПС меняется в зависимости от pH среды (в зависимости от добавления ТФУ). Для оценки влияния состава водно-ацетонитрильного элюента на удерживание исследуемых соединений использовали модели Снайдера-Сочевинского и Сочевинского-Вахтмейстера. Были рассчитаны угловые коэффициенты соответствующих моделей.

Ключевые слова: имидазолиевые ионные жидкости, сверхсшитый полистирол, обращенно-фазовая высокоэффективная жидкостная хроматография, флавоноиды, ароматические кислоты

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Introduction

Extracts of medicinal plants are complex multicomponent mixtures, the main components of which are biologically active compounds (BACs), for example, flavonoids, coumarins, aromatic acids. BACs exhibit a number of important pharmacological properties, therefore they are widely used as the main active pharmaceutical ingredients in medicines [1-3]. One of the main methods of dealing with extracts of medicinal plant raw materials is reversed-phase high-performance liquid chromatography (RP HPLC). This method is based on different sorption of substances depending on their structures on nonpolar sorbents from water-organic solutions.

The analysis of extracts of medicinal plant raw materials is difficult because many components in its composition have close physical and chemical properties of molecules, which leads to overlapping of chromatographic peaks [4]. The choice of optimal chromatographic separation conditions and the development of techniques for qualitative and quantitative determination of compounds in plant raw materials is an important and urgent task in this regard.

Alkyl-bonded silica gels are the classical and most common sorbents in RP HPLC [5-8]. However, they can work effectively in a narrow pH range. Hydrophobic microporous polymer sorbents, e.g., hypercrosslinked polystyrene (HPS), are no less promising

stationary phases in RP HPLC [9-12]. In turn, they are stable using water-acetonitrile eluents of any pH [13]. Furthermore, HPS does not contain residual silanol groups that contribute to additional blurring of chromatographic peaks [5, 14-16].

In addition to stationary phase replacement, another way to influence the efficiency of chromatographic separation is to select the optimal composition of the water-organic eluent. The nature and content of the organic solvent, as well as various additives in the water-organic eluent, are of great importance. The analysis is often carried out in the presence of buffer solutions and acids that reduce the pH of the mobile phase and inhibit the dissociation of analytes. Ionic liquids (ILs) are currently used as alternative additives to the water-organic eluent. ILs are low-temperature molten salts that have a number of unique physical and chemical properties and meet the requirements of green chemistry [17]. ILs usually consist of a bulk organic cation and an organic or inorganic anion. ILs have a dual nature, therefore they can interact with both the components of the stationary phase and the components of the mobile phase, forming ion pairs of different nature with the analyzed substances [15, 18-19]. Thus, a complex mixed retention mechanism, involving various types of interactions, can be realized in systems with ILs. A number of works are devoted to the study of the retention of alkaloids, herbicides,

aromatic acids and their isomers, antibiotics and other substances in systems with additives of ILs to the mobile phase [6, 20-25].

The aim of this work is to study the effect of additives of imidazolium ionic liquids to the eluent on the sorption of flavonoids and aromatic acids on hypercrosslinked polystyrene (HPS) by RP HPLC.

Materials and methods

Chemicals and reagents. All chemicals and reagents used were of analytical grade. Commercial standards of flavonoids and aromatic acids («Sigma-Aldrich», «abc» and «BLDpharm») were used as sorbates. Structural formulas and some physical and chemical parameters of the studied compounds are given in Table 1. Molecular parameters of sorbates were calculated by quantum chemical modeling of molecular structures followed by additional processing of optimized structures. Quantum chemical modeling was performed with Gaussian 09 software using the hybrid density functional B3LYP and the CC-pVDZ basis and taking into account the solvent, acetonitrile, by the SCRF method.

Structural formulas and some physical and chemical parameters of the used imidazolium ionic liquids are given in Table 2.

Deionized water obtained on a DME 1B membrane deionizer (CJSC «BMT», Russia) and acetonitrile, puriss. («Component reaktiv», Russia) were used to prepare mobile phases and dissolve analytes.

Chromatographic conditions. The investigations were carried out under RP HPLC conditions. All chromatographic experiments were performed in the linear region of the sorption isotherm (Henry region) using extremely dilute solutions of the studied compounds. A «Milichrom 1» liquid chromatograph with a UV spectrophotometric detector and a Varian ProStar high-pressure plunger pump were used. Detection was carried out at a wavelength of 280 nm. Hypercrosslinked polystyrene «MMN1 Purolite» (particle size of 5 μm , specific surface area of about 1000 m^2/g) was used as a sorbent.

The chromatographic column was kindly provided by Prof. V.A. Davankov. Hypercrosslinked polystyrene are characterized by the presence of micro- and mesopores [11]. A solid-state electric thermostat was used to temperature control of the column (column size 150 mm·4.6 mm). Chromatographic experiments were performed in isocratic elution mode. The volumetric flow rate of the eluent was 200 $\mu\text{L}/\text{min}$. Water–acetonitrile mixtures in different volume ratios were used as water-organic eluents. Trifluoroacetic acid (TFA) was added to create the pH of the mobile phase (pH=2.0). Commercial imidazolium ionic liquids (2.64 mmol/L) were used as specific modifiers of the water-acetonitrile solution (Table 2). Before starting the chromatographic experiment, the eluents were degassed on the ultrasonic unit «MELFIZ».

Theory and calculation. Average retention times (t_R) of sorbates from 5 parallel experiments were used. Retention factors (k) were calculated from the retention times of sorbates (t_R) and dead time (t_M) determined from the system peak. The error in the determination of retention factors was not more than 2%.

In the semi-empirical Snyder-Soczeński model, the dependence of the retention factor (k) on the composition of the binary mobile phase is represented by the following equation [26-27]:

$$\log k = a - n \cdot \log X_m \quad (1)$$

where n is a number of mobile phase molecules that are displaced by one solute molecule from adsorbent surface upon adsorption; X_m is a mole fraction of organic component (acetonitrile) in the eluent.

The semi-empirical Soczeński-Wachtmeister retention model assumes a linear relationship between the logarithm of the retention factor ($\log k$) with the volume fraction of the organic modifier (φ):

$$\log k = \log k_w - S\varphi, \quad (2)$$

where k_w is the retention factor of compounds at 100% water content in the eluent; S is angular coefficient, numerically equal to the size of the hydrophobic contact area of

Table 1. Structural formulas and some physical and chemical parameters of the studied compounds calculated by quantum chemical modeling method

Таблица 1. Структурные формулы и некоторые физико-химические параметры исследуемых соединений, рассчитанные методом квантово-химического моделирования

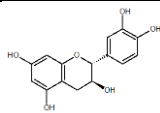
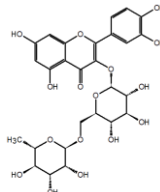
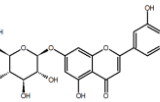
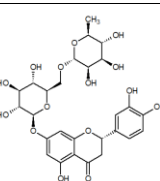
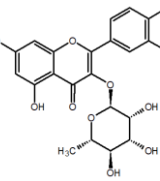
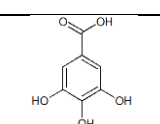
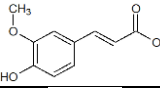
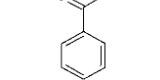
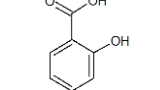
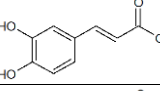
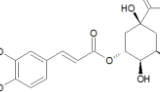
| № | Name | Structural formula | Dipole Moment (μ), Debye | Polarizability (α), Bohr ³ | Van der Waals volume (V), Å ³ | Van der Waals surface (S), Å ² |
|-----------------------|------------------|---|--------------------------------|--|--|---|
| Flavonoids | | | | | | |
| 1 | (+)-Catechin |  | 6.89 | 207.32 | 251.12 | 284.13 |
| 2 | Rutin |  | 6.47 | 484.63 | 499.75 | 547.40 |
| 3 | Cynaroside |  | 13.72 | 376.14 | 369.45 | 416.22 |
| 4 | Hesperidin |  | 11.82 | 415.26 | 520.54 | 579.41 |
| 5 | Quercitrin |  | 11.56 | 369.71 | 371.26 | 416.74 |
| Aromatic acids | | | | | | |
| 6 | Gallic acid |  | 9.41 | 148.37 | 136.75 | 165.03 |
| 7 | Ferulic acid |  | 7.10 | 222.42 | 177.42 | 213.85 |
| 8 | Benzoic acid |  | 6.55 | 123.39 | 115.14 | 140.04 |
| 9 | Salicylic acid |  | 5.27 | 105.84 | 370.07 | 422.05 |
| 10 | Caffeic acid |  | 6.11 | 202.17 | 159.09 | 192.59 |
| 11 | Chlorogenic acid |  | 7.07 | 286.07 | 300.56 | 345.90 |

Table 2. Structural formulas and some physical and chemical parameters of the used imidazolium ionic liquids

Таблица 2. Структурные формулы и некоторые физико-химические параметры используемых имидазолиевых ионных жидкостей

| № | Name | Structural formula | Mr, g/mol | Melting point, °C | LogP |
|---|---|--------------------|-----------|-------------------|------|
| 1 | 1-Butyl-2,3-dimethylimidazolium tetrafluoroborate [C ₄ dMIM][BF ₄] | | 240.05 | 38-40 | 5.30 |
| 2 | 1-Butyl-3-methylimidazolium bromide [C ₄ MIM][Br] | | 219.12 | 65-75 | 3.59 |
| 3 | 1-Hexyl-3-methylimidazolium bromide [C ₆ MIM][Br] | | 247.18 | -54.9 | 4.73 |
| 4 | 1-Decyl-3-methylimidazolium bromide [C ₁₀ MIM][Br] | | 303.28 | 30 | 7.00 |
| 5 | 1-Methyl-3-octylimidazolium bromide [MC ₈ IM][Br] | | 275.23 | -61.9 | 5.87 |

the sorbate with the surface of the sorbent, φ is volume fraction of organic modifier in the eluent [28-29].

Results and discussion

Influence of the structure of flavonoids and aromatic acids on their retention in the systems «hypercrosslinked polystyrene (HPS) – water-acetonitrile solution without and with the addition of imidazolium ionic liquids (ILs)». Figure 1 shows a comparative diagram of the change in retention factors of the studied compounds on HPS from water-acetonitrile eluents.

It was found that strongly polar acids (gallic acid, chlorogenic acid) are sorbed rather weakly on HPS. This is due to the predominance of strong specific interactions of these molecules with the components of the

water-acetonitrile eluent. Diglycoside derivatives of flavonoids are retained on HPS weaker than monoglycoside derivatives. For example, rutin is eluted much faster than quercitrin. There is no correlation between retention and their dipole moments and polarizability: the more polar and less voluminous quercitrin molecule is retained much stronger than the rutin molecule, characterized by low dipole moment and high polarizability value. A similar situation is observed for the hesperidin – cynaroside pair. It was shown that the sorption of these flavonoids is strongly influenced by the volume of the glycoside substituent, which makes an additional contribution to intermolecular interactions with the sorbent and components of the polar eluent. Probably, a size exclusion sorption mechanism of the studied com-

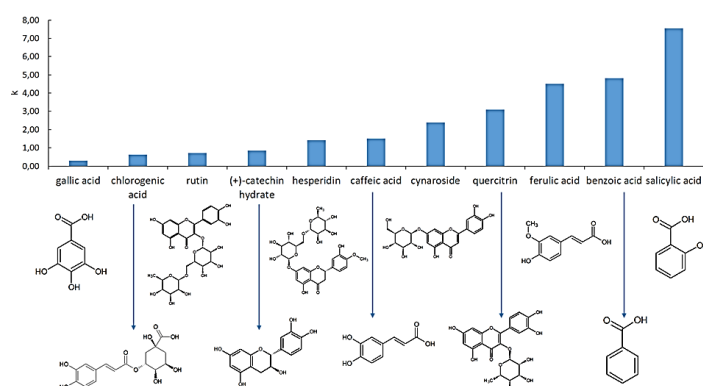


Fig. 1. Comparative diagram of flavonoids retention factors in systems without the addition of 0.1% TFA to the water-acetonitrile mobile phase (pH=5.7) depending on the structure of sorbates
Рис. 1. Сравнительная диаграмма факторов удерживания флавоноидов в зависимости от их структуры в системах с добавлением 0.1% TFA в водно-ацетонитрильную подвижную фазу (pH=5.7)

pounds on HPS cannot be excluded. However, the observed size exclusion effect is not classical. In this interpretation, it is possible to consider the effect of penetration of sorbate molecules into the nonpolar sorbent pore and subsequent interaction with the aromatic network of HPS. The networks of hypercrosslinked sorbents are rather flexible and their porous structure may easily change [12]. Dry hypercrosslinked polystyrenes tend to increase in volume on contacting with water-acetonitrile eluents. The size of pores slightly increases in swollen (by a factor of 2-3) networks [12]. We hypothesize that the nonpolar HPS surface can interact with the flavonoid molecule through both dispersion interactions and specific interactions (with hydroxyl groups (–OH) of glycosides and with delocalized π -electrons of aromatic rings). Thus, larger molecules containing diglycoside substituents will not fully penetrate into the pore of HPS, therefore, the intensity of dispersion interactions with the aromatic network of the sorbent will decrease. The nonpolar pore space will be more accessible for polar monoglycoside derivatives of flavonoids. This leads to an increase in dispersion interactions and, consequently, to an increase in retention.

Quite small molecules of ferulic acid, benzoic acid and salicylic acid are characterized by the highest retention factors in the system with HPS. They are probably able to

interact intensively and nonspecifically with the aromatic structure of HPS, in particular, in the pore space.

Influence of trifluoroacetic acid (TFA) addition on the sorption of the studied compounds in the system with HPS and imidazolium ILs. Two systems, with and without the addition of trifluoroacetic acid (TFA) to the eluent, were investigated to study the effect of imidazolium ILs on the retention of the studied flavonoids and aromatic acids on HPS. HPS is a hydrophobic and nonpolar sorbent, so the acid-free system allows us to evaluate the true effect of ILs on the sorption of the compounds.

Figure 2 presents comparative diagrams of the retention factors of sorbates in the considered systems with and without the addition of 0.1% TFA to the eluent. It was found that the introduction of imidazolium ILs leads to an increase in the retention of flavonoids (except for hesperidin and mobile phase, containing IL [C₄MIM][Br]) in the TFA-free systems in the mobile phase compared to the system without ILs and without TFA additives (Figure 2a). There is a tendency to increase the sorption of most of the studied flavonoids on HPS in the systems with bromide ILs in series [C₄MIM][Br] – [C₆MIM][Br] – [MC₈IM][Br], and decrease in the system with [C₁₀MIM][Br]. The hydroxyl groups of flavonoids can be partially ionized [30] in near neutral medium (pH=5.7

without the addition of 0.1% TFA to the mobile phase). This may lead to the formation of ionic pairs between negative flavonoid fragments and positively charged imidazole fragments of the investigated ILs. Hydrophobic ion pairs are strongly sorbed on HPS. However, the hydrophobic pair between the flavonoid and the IL [C₁₀MIM][Br] is bulky and probably cannot penetrate deep enough into the (?) HPS pore. Thus, the intensity of interactions with the aromatic sorbent network decreases. Sufficiently high retention of flavonoids is observed in the system with tetrafluoroborate IL [C₄dMIM][BF₄].

The effect of adding bromide ILs on the sorption of flavonoids in the system with 0.1% TFA (pH=2.0) in the mobile phase is slightly reduced, but some patterns are observed. It is noticed that the retention factors decrease with the addition of all bromide ILs

used compared to the system without ILs (Figure 2b). Moreover, the sorption of flavonoids is slightly higher in the system with IL [C₁₀MIM][Br] compared to other systems containing additives of bromide ILs. Thereby, it is shown that the effect of ILs on flavonoids sorption will vary greatly depending on the pH of the water-acetonitrile solution. In this case, the opposite patterns are observed. Probably, competitive sorption at the interface, as well as a hydrophobic effect reduction, will be the predominant effect of ILs in system with 0.1% TFA in the mobile phase. It is also shown that the addition of tetrafluoroborate IL [C₄dMIM][BF₄] significantly increases the sorption of flavonoids on HPS. This is probably due to the nature of the anion, which can strongly change the structural self-organization of the

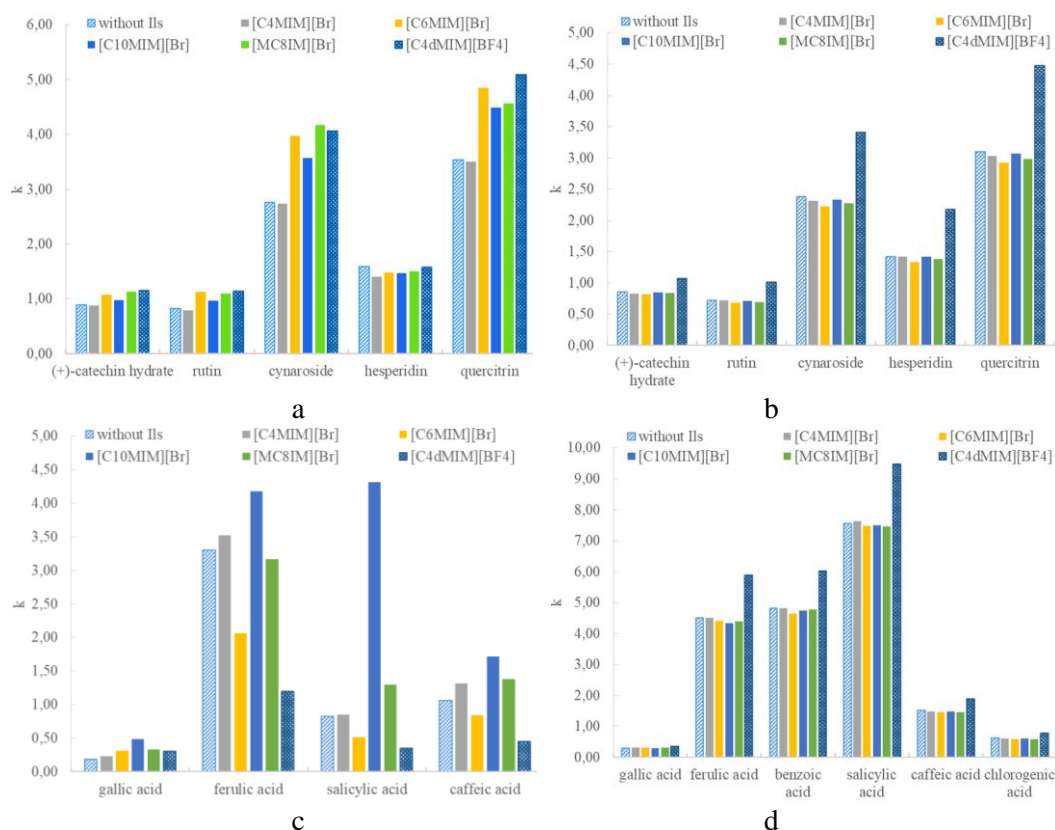


Fig. 2. Comparative diagrams of retention factors of the investigated a, b – flavonoids and c, d – aromatic acids in the considered systems with imidazolium ILs; eluent: water/acetonitrile – 70/30 vol.% a, c – without and b, d – with addition of 0.1% TFA.

Рис. 2. Сравнительные диаграммы факторов удерживания исследуемых а, б – флавоноидов и с, д - ароматических кислот в рассматриваемых системах с имидазолиевыми ИЖ; элюент: вода/ацетонитрил – 70/30 об.% а, с - без и б, д – с добавлением 0,1% TFA

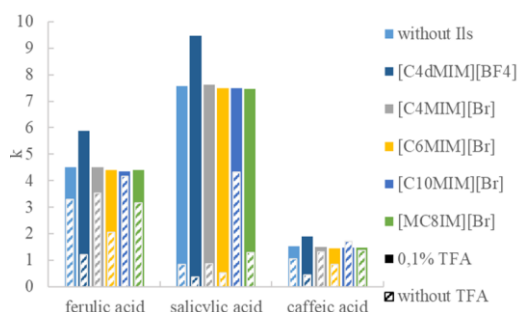


Fig. 3. Comparative diagram of retention factors of ferulic acid, salicylic acid and caffeic acid on HPS in systems without and with 0.1% TFA addition to the mobile phase.

Рис. 3. Сравнительная диаграмма факторов удерживания феруловой, салициловой и кофейной кислот на СПС в системах без и с добавлением 0,1% TFA в подвижную фазу

hydrogen bond network of the water-acetonitrile eluent and change the hydrophobic effect. This is quite evident in the acidic medium compared to the TFA-free system.

The bromide and tetrafluoroborate anions are both chaotropic, however bromide anion is more kosmotropic [31]. The IL [C₄dMIM][BF₄] contains two rather chaotropic ions in its structure. Therefore, the retention in this system should be comparable or slightly lower than in the system with bromide IL under the same conditions. Tetrafluoroborate anion will affect the conformation of the aromatic network of HPS by penetrating inside the pores and destructuring the pore fluid. Exposure of aromatic fragments of HPS may occur due to this fact. This leads to the appearance of additional active centers of flavonoid sorption.

Aromatic acids are also retained differently on HPS in the systems with imidazolium ILs depending on the presence of TFA in the eluent (Figure 2c, Figure 2d). It was found that aromatic acids are strongly retained in the system with IL [C₁₀MIM][Br] in the absence of TFA in the mobile phase (Figure 2c). This is probably due to the formation of hydrophobic ionic pairs between positively charged imidazole fragment of these ILs and negatively charged carboxyl groups of the aromatic acids. This leads to an increase in their sorption on HPS. Chlorogenic acid begins to retain on HPS only in systems with ILs [MC₈IM][Br] and [C₁₀MIM][Br]. This fact can confirm the possibility of formation of hydrophobic ion

pairs and realization of ion-pair mechanism of acids retention on HPS in the systems with some used bromide ILs. The lowest aromatic acids retention is observed in the system with tetrafluoroborate IL [C₄dMIM][BF₄].

The effect of bromide ILs on the retention of aromatic acids on HPS is slightly reduced in systems with 0.1% TFA (Figure 2d). The addition of 0.1% TFA to the water-acetonitrile eluent converts all aromatic acids to the neutral form. Thus, the direct effect of ILs on sorbates is reduced. The formation of ion pairs is unlikely in this case. Competitive sorption of bromide ILs and sorbates at the interface is more probable. In this connection there is a slight decrease in the retention of aromatic acids compared to the systems without ILs. A significant increase in acids retention in the system with tetrafluoroborate IL [C₄dMIM][BF₄] is observed. The explanation of this effect is discussed above.

The addition of 0.1% TFA to the water-acetonitrile eluent significantly affects the retention patterns of ferulic acid, caffeic acid and salicylic acid. Figure 3 shows a comparative diagram of the retention factors of these three acids in different systems under consideration.

It was found that salicylic acid retains more strongly than the other studied aromatic acids in all chromatographic systems with the addition of 0.1% TFA to the eluent. If the water-acetonitrile eluent does not contain 0.1% TFA (near neutral medium, pH=5.7), salicylic acid begins to retain

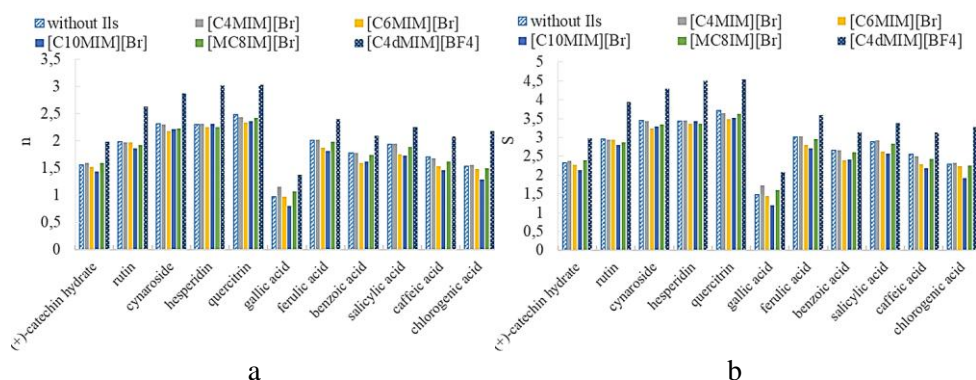


Fig. 4. Comparative diagram of angular coefficients for studied flavonoids and aromatic acids obtained from the a – Snyder–Soczewiński and b – Soczewiński–Wachtmeister models for investigated systems containing imidazolium ILs.

Рис. 4. Сравнительная диаграмма угловых коэффициентов, полученных в рамках модели а – Снайдера-Сочевинского и б – Сочевинского-Вахтмейстера, для изучаемых флавоноидов и ароматических кислот в системах, содержащих добавки имидазолиевых ИЖ.

weaker than ferulic acid and caffeic acid in almost all systems (except for the system with $[C_{10}MIM][Br]$). It is most likely that specific electron density delocalization is realized in dissociated forms of ferulic acid and caffeic acid. Therefore, there is a possibility to realize additional specific interactions with the HPS network and, accordingly, to enhance sorption. This effect is not observed for salicylic acid even with the possible formation of intramolecular H-bond in this molecule (between carboxyl and hydroxyl groups). For the system with IL $[C_{10}MIM][Br]$, patterns similar to those found in systems with 0.1% TFA added to the mobile phase are observed. The bulk imidazolium cation of this IL probably forms a thermodynamically stable ion pair with these aromatic acids. This prevents the occurrence of specific electron density delocalization in molecules of ferulic and caffeic acid.

Influence of water-acetonitrile eluent composition on the chromatographic retention of studied compounds. The Snyder-Soczewiński and Soczewiński-Wachtmeister models were used to study in more detail the effect of imidazolium ILs on the chromatographic retention of the investigated flavonoids and aromatic acids on HP. Concentration dependences of retention factors are more informative when analyzing the retention of sorbates in different systems. Table 3

contains the equations obtained within the models under consideration. The obtained linear equations are characterized by excellent correlation.

Figure 4 shows comparative diagrams of the angular coefficients (n and S) found from the equations derived from the Snyder-Soczewiński and Soczewiński-Wachtmeister retention models.

It was found that the angular coefficients vary greatly depending on the structure of the ILs used. This is particularly pronounced for aromatic acids. For example, the angular coefficients decrease with the addition of bromide ILs to the eluent, compared to the system without ILs additives. A regularity of decreasing angular coefficients with increasing alkyl chain length at the same substituents location in the IL cation is observed ($[C_4MIM][Br]$ – $[C_6MIM][Br]$ – $[C_{10}MIM][Br]$). The system with the addition of tetrafluoroborate IL $[C_4dMIM][BF_4]$ is characterized by rather high sorbates angular coefficients within the corresponding models. This seems to be directly related to the nature of the anion, which is able to affect the HPS network and increase the number of active sorption centers for aromatic acids. A similar trend is observed for flavonoids, but it is not so pronounced.

Table 3. Values of angular coefficients and coefficients of determination obtained within the Snyder–Soczewiński and Soczewiński–Wachtmeister retention models (acetonitrile concentration range 30÷50 vol.%, 0.1 % TFA, $t=65^{\circ}\text{C}$)

Таблица 3. Значения угловых коэффициентов, полученных в рамках модели Снайдера-Сочевинского и Сочевинского-Вахтмейстера (диапазон содержания ацетонитрила 30-50 об.%, 0.1% TFA, $t=65^{\circ}\text{C}$)

| №* | Snyder–Soczewiński | | Soczewiński–Wachtmeister | | Snyder–Soczewiński | | Soczewiński–Wachtmeister | |
|---------------------------|--------------------|----------------|--------------------------|----------------|---|----------------|--------------------------|----------------|
| | n | R ² | S | R ² | n | R ² | S | R ² |
| Without ILs | | | | | [C ₄ dMIM][BF ₄] | | | |
| 1 | 1.55 | 0.99 | 2.31 | 0.99 | 1.99 | 0.99 | 2.98 | 0.99 |
| 2 | 1.99 | 0.99 | 2.96 | 0.99 | 2.63 | 0.99 | 3.94 | 0.99 |
| 3 | 2.31 | 0.99 | 3.44 | 0.98 | 2.89 | 0.99 | 4.30 | 0.99 |
| 4 | 2.30 | 0.98 | 3.43 | 0.97 | 3.01 | 0.99 | 4.50 | 0.99 |
| 5 | 2.49 | 0.99 | 3.71 | 0.99 | 3.04 | 0.99 | 4.54 | 0.99 |
| 6 | 0.97 | 0.83 | 1.49 | 0.87 | 1.370 | 0.96 | 2.07 | 0.98 |
| 7 | 2.01 | 0.99 | 3.01 | 0.99 | 2.40 | 0.99 | 3.60 | 0.99 |
| 8 | 1.78 | 0.99 | 2.66 | 0.99 | 2.09 | 0.99 | 3.13 | 0.99 |
| 9 | 1.93 | 0.99 | 2.89 | 0.99 | 2.25 | 0.99 | 3.37 | 0.99 |
| 10 | 1.70 | 0.99 | 2.55 | 0.99 | 2.09 | 0.99 | 3.13 | 0.99 |
| 11 | 1.53 | 0.99 | 2.29 | 0.99 | 2.18 | 0.99 | 3.28 | 0.99 |
| [C ₄ MIM][Br] | | | | | [C ₆ MIM][Br] | | | |
| 1 | 1.59 | 0.99 | 2.38 | 0.99 | 1.52 | 0.99 | 2.27 | 0.99 |
| 2 | 1.97 | 0.99 | 2.94 | 0.99 | 1.97 | 0.99 | 2.95 | 0.99 |
| 3 | 2.30 | 0.99 | 3.44 | 0.99 | 2.17 | 0.99 | 3.24 | 0.99 |
| 4 | 2.31 | 0.99 | 3.45 | 0.99 | 2.25 | 0.99 | 3.36 | 0.99 |
| 5 | 2.44 | 0.99 | 3.65 | 0.99 | 2.34 | 0.99 | 3.49 | 0.99 |
| 6 | 1.15 | 0.99 | 1.73 | 0.99 | 0.98 | 0.98 | 1.49 | 0.96 |
| 7 | 2.02 | 0.99 | 3.02 | 0.99 | 1.87 | 0.99 | 2.79 | 0.99 |
| 8 | 1.77 | 0.99 | 2.65 | 0.99 | 1.60 | 0.99 | 2.39 | 0.99 |
| 9 | 1.95 | 0.99 | 2.92 | 0.99 | 1.76 | 0.99 | 2.62 | 0.99 |
| 10 | 1.67 | 0.99 | 2.51 | 0.99 | 1.53 | 0.99 | 2.29 | 0.99 |
| 11 | 1.55 | 0.99 | 2.32 | 0.99 | 1.49 | 0.99 | 2.23 | 0.99 |
| [C ₁₀ MIM][Br] | | | | | [MC ₈ IM][Br] | | | |
| 1 | 1.43 | 0.99 | 2.13 | 0.98 | 1.60 | 0.99 | 2.39 | 0.99 |
| 2 | 1.87 | 0.99 | 2.80 | 0.99 | 1.92 | 0.99 | 2.87 | 0.99 |
| 3 | 2.21 | 0.99 | 3.30 | 0.98 | 2.23 | 0.99 | 3.34 | 0.99 |
| 4 | 2.31 | 0.99 | 3.44 | 0.98 | 2.25 | 0.99 | 3.36 | 0.99 |
| 5 | 2.36 | 0.99 | 3.52 | 0.99 | 2.42 | 0.99 | 3.62 | 0.99 |
| 6 | 0.80 | 0.98 | 1.20 | 0.96 | 1.07 | 0.99 | 1.61 | 0.99 |
| 7 | 1.81 | 0.99 | 2.70 | 0.99 | 1.98 | 0.99 | 2.96 | 0.99 |
| 8 | 1.62 | 0.99 | 2.41 | 0.99 | 1.74 | 0.99 | 2.61 | 0.99 |
| 9 | 1.73 | 0.99 | 2.57 | 0.99 | 1.89 | 0.99 | 2.83 | 0.99 |
| 10 | 1.47 | 0.99 | 2.19 | 0.99 | 1.62 | 0.99 | 2.42 | 0.99 |
| 11 | 1.29 | 0.99 | 1.92 | 0.99 | 1.50 | 0.99 | 2.25 | 0.99 |

Conclusions

In this work the chromatographic behavior of some aromatic acids and flavonoids on hypercrosslinked polystyrene (HPS) in the systems with water-acetonitrile eluents containing imidazolium ionic liquids (ILs) additives was studied by reversed-phase HPLC

(RP HPLC). It was shown that the mechanism of action of imidazolium ILs additives on the sorption of studied compounds depends on the pH of the mobile phase. It was found that the ion-pair mechanism of separation of the studied sorbates is possible in neutral medium. This is due to the possible formation of stable ionic pairs between molecules of sorbates (flavonoids and aromatic

acids) and the cation of imidazolium IL. In acidic medium, retention is probably due to competitive sorption between sorbate molecules and ILs. All investigated compounds are strongly sorbed in the eluent with the addition of 0.1% TFA and tetrafluoroborate IL ([C₄dMIM][BF₄]). The semi-empirical Snyder-Soczewiński and Soczewiński-Wachtmeister retention models were considered. It is noticed that the angular coefficients *n* and *S* of the corresponding models decrease with the addition of bromide ILs to the eluent compared to the system without ILs additives. The decrease in the angular

coefficients for aromatic acids in systems with bromide ILs is observed in the following series: [C₄MIM][Br] – [C₆MIM][Br] – [C₁₀MIM][Br]. The system with tetrafluoroborate IL [C₄dMIM][BF₄] is characterized by rather high angular coefficients of sorbates.

Conflict of interest

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

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