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A natural deep eutectic solvent - protonated L-proline-xylitol - based stationary phase for gas chromatography

Malwina Momotko[a,*], Justyna Łuczak[a,c], Andrzej Przyjazny[b], Grzegorz Boczkaj[c,d,*]

[a] Gdansk University of Technology, Faculty of Chemistry, Department of Process Engineering and Chemical Technology, 80 – 233 Gdansk, G. Narutowicza St. 11/12, Poland
[b] Kettering University, 1700 University Avenue, Flint, MI 48504, USA
[c] Advanced Materials Center, Gdansk University of Technology, 80 – 233 Gdansk, G. Narutowicza St. 11/12, Poland
[d] Gdansk University of Technology, Faculty of Civil and Environmental Engineering, Department of Sanitary Engineering, 80 – 233 Gdansk, G. Narutowicza St. 11/12, Poland

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ABSTRACT

The paper presents a new kind of stationary phase for gas chromatography based on deep eutectic solvents (DES) in the form of a mixture of L-proline (protonated with hydrochloric acid) as a hydrogen bond acceptor (HBA) and xylitol as a hydrogen bond donor (HBD) in a molar ratio of HBA:HBD 5:1. DES immobilized on a silanized chromatographic support was tested by gas chromatography (GC) in order to determine its resolving power for volatile organic compounds. Studies have demonstrated the suitability of this type of DES as a stationary phase for GC. The Rohrschneider-McReynolds constants were determined for the synthesized DES, revealing that it is a polar stationary phase ($\Sigma(\Delta I) = 1717$). The selectivity of DES is influenced by the presence of hydroxyl groups in the xylitol structure capable of forming hydrogen bonds of a donor nature and the proton acceptor properties of the protonated L-proline structure. The presence of additional interactions is brought about by the presence of the carboxyl group. As a result, the retention of alcohols is several times higher (200-670%) than the expected value based on their boiling points. A significant increase in retention (400-970%) was also found for pyridine derivatives. The developed DES stationary phase is characterized by very good repeatability of retention and stability (up to 140°C). The efficiency of the prepared columns (6300-11300 theoretical plates/m) and the selectivity of the DES stationary phase are competitive with the commercial products.

1. Introduction

Deep eutectic solvents have recently been the subject of very extensive research on their applications in various areas of science and technology. The possibility of synthesizing DESs showing specific intermolecular interactions with numerous groups of chemical compounds constitutes their great potential in separation techniques, such as extraction media [1,2], absorbents [3] and membrane components [4–6]. Also in chromatographic techniques, DES gained high attention [7]. In literature, often a term such as DES based stationary phases is used. However, analysis of such reports reveals that DESs are not involved in final stage, i.e. during application of such materials in separation techniques. Mostly, they were used at synthesis stage (as solvents or additives) to obtain materials with improved properties, such as modified silica gel [8–12]. In other studies, it was claimed that addition of DES provide improved dispersion of nanomaterials subjected for functionalization [13,14]. DESs proved to be useful (as specific pore size formers) during synthesis of packings for size exclusion chromatography (SEC) [15–18]. In other approaches, DES is present during polymerization stage, resulting in formation of new sorptive material [19,20]. It is clear that obtained stationary phases are not liquid and primary DES does not possess its “liquid” properties in final material. It is worth to mention, that in most attempts, the final material lacks of DES components. In terms of liquid chromatography (LC), DESs were used as additives to mobile phase [21,22] as well as main mobile phase component [23,24]. Still, the literature about application of DESs as stationary phases is scarce.

So far, it has been shown that DES can be used in the form of a mixture of heptadecanoic acid and tetrabutylammonium chloride in a mole ratio of HBD:HBA 2:1 as a stationary phase for gas chromatography (GC) [25]. The obtained columns were character-

* Corresponding author.
E-mail addresses: malwina.momotko@wp.pl (M. Momotko), grzegorz.boczkaj@pg.edu.pl (G. Boczkaj).

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ized by good efficiency and stability of retention parameters during long-term use. The McReynolds constant values for the synthesized DES phase were compared with the literature values for commercially available stationary phases, revealing that a material with a different selectivity was obtained. The sum of the McReynolds constants is equal to 1174 which indicates that the synthesized DES stationary phase has a medium polarity. The results of this work expanded the applicability of deep eutectic solvents. The possibility of modifying the properties of DES by changing one of its components or by adding an extra solute offer great opportunities for the preparation of new stationary phases.

Countless combinations of HBA and HBD allow preparation of DESs having high, often unique, selectivity due to the complex nature of sorption interactions between DES and the separated compounds. The use of DESs in many areas of separation techniques is possible due to the similarity of their physicochemical properties to those of classic solvents. However, in many cases DESs are prepared from volatile or thermally labile compounds, precluding most of them as potential components for stationary phases for GC. As a result of these limitations, DESs obtained using protonated L-proline as the hydrogen bond acceptor (HBA) and xylitol as the hydrogen bond donor (HBD) were selected for this study. As both components have a natural origin, this type of DES can be named as Natural Deep Eutectic Solvents (NADES) [24]. Preliminary studies were performed with DESs synthesized using L-proline protonated with three different acids (hydrochloric, sulfuric and phosphoric). Subsequently, the DES stationary phase based on L-proline protonated with hydrochloric acid was selected for further work, as it turned out to be the only one suitable as a stationary phase for GC.

2. Materials and methods

2.1. Materials

The investigated DES (L-proline, xylitol) was prepared from reagents of >99% purity (Sigma Aldrich, Burlington, USA) while hydrochloric, phosphoric and sulfuric acid were of analytical reagent grade (POCH, Gliwice, Poland). A Chromosorb W AW-DMCS (80/100 mesh) chromatographic support (Johns-Manville, Denver, United States) was used as DES support. DES was deposited on the support from methanol (analytical reagent, POCH, Gliwice, Poland). A thin-walled steel tubing (1/8" ID, L = 2.70 m) were used as packed columns. A silanized glass wool (Supelco, Bellefonte, USA) was used to hold the stationary phase in the columns (small portions of the wool were placed at the both ends of the column).

Standard test mixtures of volatile organic compounds (VOCs) (Sigma Aldrich, Burlington, USA) and a mixture of n-alkanes in the range of n-C_5 to n-C_{17} (Analytical Controls, Houston, USA) were used in the GC tests of the obtained columns. Typically, test compounds were dissolved in carbon disulfide (analytical reagent, Merck, Darmstadt, Germany). The gases in the GC analysis included nitrogen (N5.0, Linde Gas, Gdynia, Poland) as the mobile phase (carrier gas), while the FID detector gases were hydrogen (N5.5, from a hydrogen generator) and air (N5.0, Linde Gas).

2.2. Apparatus

All chemicals were precisely prepared on the weight basis using a AS.310.R2 analytical balance (Radwag, Radom, Poland). A 06-MSH-PRO-T magnetic stirrer (Chemland, Stargard, Poland) was used to prepare DESs and their solutions in methanol. A Rotavapor R-300 rotary evaporator (Buchi, Flawil, Switzerland) was employed to immobilize the DES on the support. To pack the columns under vacuum assistance a CRVpro4 vacuum pump (Welch, Ilmenau, Germany) was connected to one end of the column plugged with silanized wool. A Clarus 500 gas chromatograph (Perkin Elmer, Waltham, USA) with a split/splitless injection port and a flame ionization detector (FID) was used for all GC studies performed in this paper. The GC instrument was equipped with an autosampler. A PGX-H2 500 generator (Perkin Elmer, Waltham, USA) was used as hydrogen supply.

2.3. Procedure

2.3.1. Synthesis of DES

The synthesis of the deep eutectic solvent involved dissolving L-proline in an acid solution (the amount of acid with respect to L-proline was equimolar). Next, a predetermined amount of xylitol was added to the solution. Thus prepared solution was placed in a rotary evaporator and water was distilled off under reduced pressure. For the selected DES composition, two batches of DES were independently synthesized to evaluate the repeatability of their properties. Studies on the synthesis of this type of DESs and their physicochemical characteristics were the subject of a separate paper [26].

2.3.2. Preparation of DES based stationary phases and GC columns

Decantation from methanol was used to remove subsized particles from commercial Chromosorb W-AW-DMCS 80-100 mesh (Johns-Manville, Denver, United States). Subsequently, the support was dried in a rotary evaporator (removal of methanol) and activated in a vacuum oven at 230°C. The activation stage lasted 240 minutes. After the support was cooled to about 30°C, it was taken out of the oven and instantly added to a flask containing homogeneous mixture of DES and methanol (1 g DES in 150 mL of methanol). The suspension was equilibrated by mixing in a rotary evaporator for 30 min followed by evaporation of the solvent.

The stationary phase prepared in this way was then used to pack GC columns using the dry pack method. Following the packing, each prepared column was conditioned at 30°C in the flow of inert gas (nitrogen, 40 mL/min) for 1 hour. Next, the column temperature was ramped from 30°C to 110°C at 1°C/min. Finally, the column outlet was joined with the flame ionization detector and the column was thermostated in 110°C. This conditions were maintained until stable signal from the detector was observed.

2.3.3. Characterization of the DES based GC stationary phase

Test standards solutions (*ca.* 500 ppm) in CS_2 were used in the studies. A methane (a 10 ppm mixture in nitrogen) was used to determine dead time.

Splitless mode was used in all injections. The injection volume was 1 µL for all solutions and 0.1 mL for gas mixtures. Linear velocity of the carrier gas was 4.21 cm/s. Both the injection port and FID were kept at 300°C. A temperature program was used in all chromatographic separations. The initial oven temperature was 35°C (held for 2 min), followed by a ramp to a final temperature (110°C) with the rate of increase of 5°C/min. The final oven temperature was held for 20 min.

Retention times and the number of theoretical plates (**N**) for standards were determined from the GC-FID chromatograms using TotalChrom v.6.3 software (Perkin Elmer, Waltham, USA). Retention (**k**) and selectivity factors ($\boldsymbol{\alpha}$) were then calculated from the obtained values. The obtained retention times ($\mathbf{t_{r,real}}$) were next used to compare them with predicted retention time values for a nonpolar stationary phase – they were calculated using boiling point (**bp**) values of the analytes ($\mathbf{t_{r,bp}}$). The predicted retention time values were obtained on the basis of determined relationship between retention time values and boiling point values ($\mathbf{t_{r,bp} = f(bp)}$) for n-alkanes used as test probes. The obtained data were also used to calculate the relative percent deviation of the retention time values (R%).

Additionally, based on protocol proposed by Davis, an interaction coefficients ($\mathbf{I_p}$) were determined [27]. The I_p was obtained as the calculated difference between $\mathbf{t_{r,real}}$ and the theoretical retention for a specified substance (this value was calculated according to equation (1)).

$$I_p = logt_{r,real,i} \cdot 100 - (A \cdot M_i + B) \quad (1)$$

where:

A- the slope of calibration curve for n-alkanes in the form i $100^{*}\log(t_r) = f(M)$

B- the intercept of calibration curve for n-alkanes in the form $100^{*}\log(t_r) = f(M)$ $\log(t_{r,real,i})$ – common logarithm of retention time of analyte *i*

M_i – molar mass of analyte *i* [g/mol]

In this way, the retention deviations were determined both with respect to boiling point value as well as the molar mass of the analytes.

2.3.4. Evaluation of stability of DES based stationary phase and repeatability of manufactured columns

Repeatability and stability of column performance were evaluated using retention time values obtained as triplicate analysis of the same mixture analyzed in given column (analysis-to-analysis) and after 50 chromatographic runs, respectively.

Column-to-column repeatability was determined by comparing retention characteristics of two independently prepared columns. For each column, an independent synthesis of DES at identical conditions was performed. In this case comparison of McReynolds constants was used as evaluation criteria.

3. Results and discussion

3.1. Suitability of DESs as stationary phases for GC

The search for new applications of DESs suitable to be used as stationary phase for GC faces considerable challenges. At present, the synthesis of new types of DESs is highly probable – the number of potential HBA and HBD is huge and so far many possible combinations have not been studied yet. However, the criteria that DESs must meet to be used as stationary phases for GC narrow these possibilities. Obviously, volatile compounds cannot be used and should be removed from consideration. The second criterion is thermal stability – depending on the applications of the developed stationary phase. In the case of separation of volatile organic compounds (VOCs), satisfactory operating temperatures of the columns are in the range of 30-100 °C. On the other hand, in order to ensure effective separation of polycyclic aromatic hydrocarbons, the required thermal stability of the stationary phase should be at least 250 °C. For these reasons, the spectrum of chemicals that can form DESs and meet the above requirements is already very limited.

DES, whose components (Fig. 1) meet the above requirements, were selected for this study. L-proline (melting point/thermal decomposition 205-228°C) [26] protonated with three different mineral acids (sulfuric, phosphoric and hydrochloric) was used as HBA and xylitol (melting point 92°C) was used as HBD in a mole ratio HBA:HBD - 5:1. Studies on the synthesis of DESs based on protonated L-proline have shown that DES can be obtained for each of the three acids listed above [26]. It is worth to mention, that according to previous studies [26], protonation of L-proline is mandatory to obtain this type of DESs. Pristine L-proline does not form DESs with xylitol. All three mineral acids mentioned above, provide successful protonation of proline, which in this form acts as HBA and forms DESs with Xylitol.

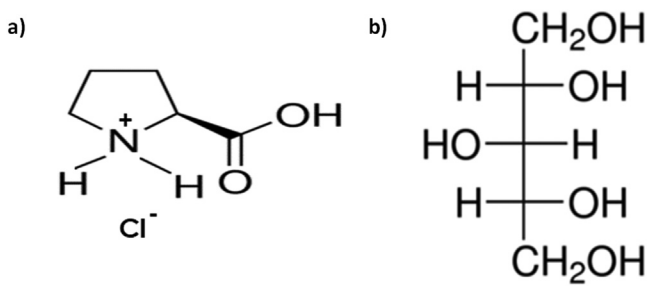

Fig. 1. Structural formulas of chemical compounds used for synthesis of DES a) protonated by hydrochloric acid L-proline b) xylitol.

During preliminary investigations, GC columns based on all three DESs were prepared. However, the initial testing of the synthesized phases revealed that in the case of the phases obtained by protonating L-proline with sulfuric and phosphoric acid, there is a considerable tailing of chromatographic peaks and a low efficiency of the columns prepared. On the other hand, in the case of DES obtained using L-proline protonated with hydrochloric acid, symmetrical chromatographic peaks and very good column efficiency were observed. This is an important observation, worth verification during future attempts to synthesize stationary phases for GC based on HBA obtained by protonation of the amino group with mineral acids.

With the given composition (HBA-HBD 5:1) and using the protonation of L-proline with hydrochloric acid, a clear colorless eutectic liquid is formed with a melting point of -37°C [26].

Chromatographic assays carried out using the synthesized GC stationary phase allowed determination of typical properties of stationary phases with respect to volatile organic compounds differing in functional groups and volatility as well as saturated hydrocarbons (n-alkanes) commonly used in GC as reference compounds. n-Alkanes do not show specific interactions with the stationary phase, and their retention depends only on their volatility. In this way, it can be determined – on the basis of the increased retention of individual VOCs – whether the tested stationary phase allows for specific sorptive interactions with test substances having specific functional groups.

3.2. Characteristics of prepared packed column and selectivity of DES stationary phase

Columns used in this study were 2.7 m long and their diameter was 1/8". The DES used in this study was completely soluble in methanol, which provided good conditions for DES immobilization on the chromatographic support using a static technique during solvent evaporation from the suspension in a rotary evaporator. The DES content of 10% (w/w) of the support was used. This is a commonly used loading amount of stationary phase for this type of chromatographic support. No aggregations of the particles or deposits on the inner walls of the round-bottom flask were observed. DES coating onto the support and packing of the column were handled the same way as are typical liquid stationary phases for GC. The column packing was added into the column in small portions assuring a free fall of the particles in the column.

The efficiency of the obtained packed columns was determined for undecane (n-C_{11}) and dodecane (n-C_{12}) as the number of theoretical plates and equal to 17072 and 30635, respectively, which should be considered a very good result for packed columns.

Examples of separation of mixtures of *n*-alkanes and sulfides are shown in Figs. 2 and 3. Fundamental physicochemical properties and retention parameters for the analytes tested are listed in Table 1.

The inspection of the data compiled in Table 1 reveals that the obtained stationary phase based on DES has an interesting selectivity, which can be interpreted by the possible sorption interactions with compounds forming DES. Protonated proline can ex-

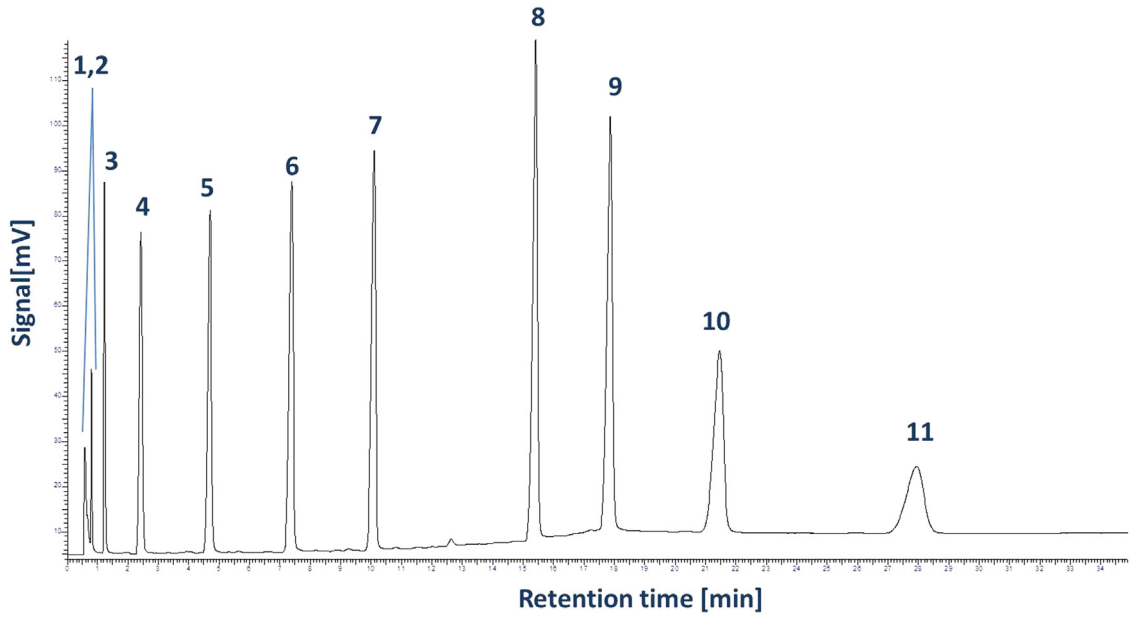

Fig. 2. Separation of n-alkanes (mixture containing *ca.* 8.3% of each standard from *n*-pentane to *n*-heptadecane without *n*-tridecane in carbon disulfide, chromatographic conditions as described in section 2.5). Injection volume 1 µL in splitless mode. Temperature program: 35°C (held for 2 min.), then ramped to 110°C at 5°C/min. 1 – *n*-C_5/*n*-C_6; 2 – *n*-C_7; 3- *n*-C_8; 4- *n*-C_9; 5- *n*-C_{10}; 6- *n*-C_{11}; 7- *n*-C_{12}; 8- *n*-C_{14}; 9- *n*-C_{15}; 10- *n*-C_{16}; 11- *n*-C_{17}.

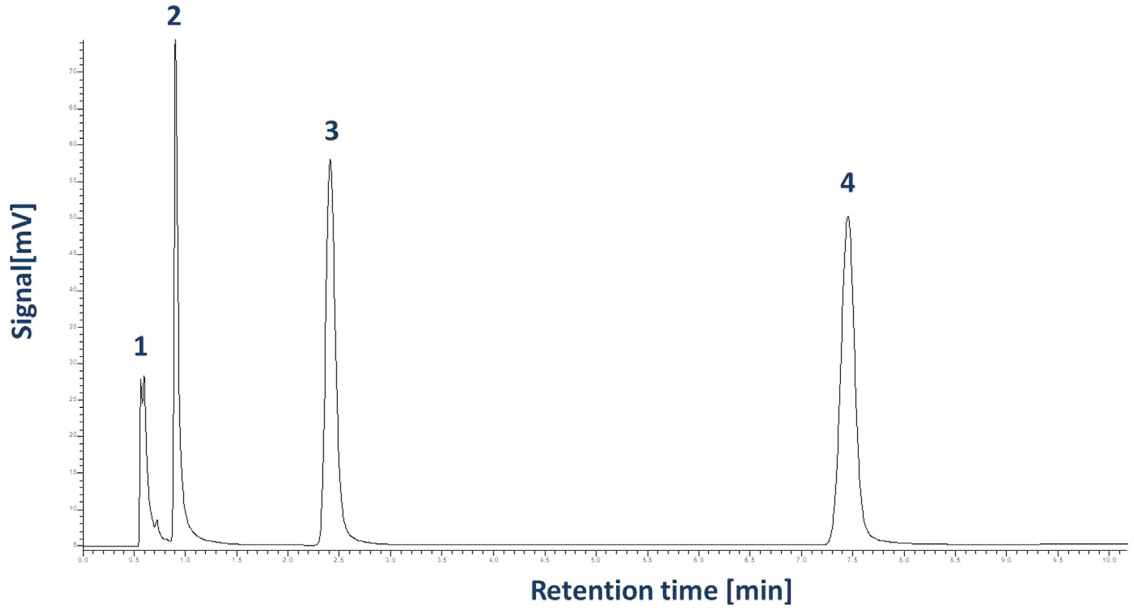

Fig. 3. Separation of sulfides (mixture containing *ca.* 0.5% of each standard in carbon disulfide (CS_2), chromatographic conditions as described in Section 2.5). Injection volume 1 µL in splitless mode. Temperature program: 35°C (held for 2 min.), then ramped to 110°C at 5°C/min. 1 – CS_2; 2 – dimethyl disulfide; 3- dipropyl sulfide; 4- dibutyl sulfide.

hibit strong proton-acceptor interactions (which is obvious due to its role as HBA in DES), but also proton-donor (presence of O-H group in carboxyl group of proline, protonated nitrogen atom in proline) and n-π interactions (presence C=O group in carboxyl group of proline) and, to a lesser extent, dispersion interactions. On the other hand, xylitol should exhibit primarily interactions characteristic of the hydroxyl group. The resultant selectivity of the DES used in GC, however, is not obvious, as the mole ratio of L-proline to xylitol (5:1) of the eutectic mixture indicates that each hydroxyl group of the xylitol molecule should interact with one protonated L-proline molecule. Then, the carboxyl group of L-proline would be expected to be responsible for the interaction with the analytes being separated. The stationary phase, however, is a liquid and it should be expected that the instantaneous form of DES and the arrangement of molecules in space can deviate from the assumed form – the existence of DES is mainly based on hydrogen bonding interactions and the presence of analyte molecules will result in competitiveness of these compounds with the DES components. In a sense, the separation mechanism can be compared to that taking place in liquid chromatography – where eluent components and analytes compete for the access to active sites of the stationary phase. This again demonstrates the enormous potential of DESs as

Table 1
Retention data of volatile organic compounds tested on DES.

b.p. [°C]	M [g/mol]	Compound	tr[min]	k	t_r theor.[min]	Δt_r[%]	I_p
		Aromatic hydrocarbons					
80.1	78.11	Benzene	0.71	0.3	0.50	66.64	42.87
110.6	92.14	Toluene	1	0.8	1.07	45.07	-6.68
136	106.17	Ethylbenzene	1.7	2.0	2.09	25.98	-18.63
144	106.17	*o*-Xylene	0.99	0.8	2.53	26.04	-60.79
159	120.19	Propylbenzene	3.5	5.3	3.50	18.49	-0.13
183	134.22	Butylbenzene	6.07	9.8	5.55	30.11	9.41
		Alcohols					
97	60.06	1-Propanol	3.35	5.0	0.74	355.58	-*
98	74.12	2-Butanol	5.8	9.4	0.76	670.36	96
102	88.15	*t*-Amyl alcohol	5.01	7.9	0.84	494.15	86
116	74.12	1-Butanol	6.11	9.9	1.24	391.23	98
119	88.15	2-Pentanol	6.8	11.2	1.35	406.14	100
138	88.15	1-Pentanol	8.8	14.6	2.19	299.03	111
156	102.17	1-Hexanol	11.9	20.2	3.29	260.96	115
160	100.16	Cyclohexanol	10.6	17.9	3.58	195.44	113
175	116.2	1-Heptanol	14.3	24.6	4.80	198.22	102
205	108.14	Benzyl alcohol	29.8	52.2	7.99	273.30	147
		Ketones					
92	86.13	Methyl isopropyl ketone	1.04	0.9	0.64	61.24	19
101	86.13	2-Pentanone	1.24	1.2	0.82	51.18	27
124	114.19	Diisopropyl ketone	1.9	2.3	1.54	20.64	18
127.6	100.16	2-Hexanone	2.47	3.4	1.69	45.89	50
131	84.12	Cyclopentanone	2.05	2.7	1.85	11.05	50
140	100.12	Acetylacetone	2.30	3.1	2.30	0.00	47
147	114.19	3-Heptanone	4.24	6.6	2.70	56.82	54
155.6	98.14	Cyclohexanone	3.71	5.6	3.26	13.68	68
169	112.17	3-Methylcyclohexanone	6.05	9.8	4.28	41.29	72
202	101.24	Acetophenone	10.29	17.4	7.62	35.05	80
		Thiophene and its alkylated derivatives					
84	84.14	Thiophene	0.74	0.3	0.54	38.21	5
112	98.17	2-Methylthiophene	1	0.8	1.11	-10.24	13
116	98.17	3-Methylthiophene	1.07	0.9	1.24	-13.97	16
133	112.19	2-Ethylthiophene	1.7	2.0	1.94	-12.40	17
		Sulfides and disulfides					
91	90.19	Diethyl sulfide	0.81	0.4	0.63	28.78	6
110	94.2	Dimethyl disulfide	0.9	0.6	1.05	-14.60	9
152	118.24	Diethyl disulfide	2.47	3.4	3.02	-18.22	22
142	122.25	Dipropyl sulfide	2.39	3.3	2.41	-0.86	12
188	146.29	Dibutyl sulfide	7.45	12.3	6.05	23.09	14
193	150.31	Dipropyl disulfide	7.35	12.1	6.59	11.61	8
200	178.36	Di-*t*-butyl disulfide	8.25	13.7	7.38	11.77	-14
		Thiols					
125	104.21	1-Pentanethiol	1.74	2.1	1.58	9.94	29
152	118.24	1-Hexanethiol	3.62	5.5	3.02	19.85	38
168.3	110.18	Thiophenol	6.69	10.9	4.22	58.37	82
174	132.27	1-Heptanethiol	6.25	10.2	4.71	32.70	33
240.56	174.35	1-Decanethiol	14.7	25.3	13.20	11.34	14
		Pyridine and derivatives					
115.2	79.1	Pyridine	13.1	22.3	1.22	972.44	110
144	93.13	3-Methylpyridine	12.3	20.9	2.53	385.92	72
145	93.13	4- Methylpyridine	16.3	28.1	2.58	531.65	85
159	107.07	2,4- Dimethylpyridine	17.6	30.3	3.50	400.77	64
		Other compounds					
132	89.09	Nitropropane	2.4	3.4	1.89	28.90	12
202	123.11	4-Methylbenzaldehyde	26.2	45.8	7.62	243.73	65
211	120.15	Nitrobenzene	22.5	39.1	8.75	156.77	61

*value of the molar mass used for the calculation of the parameter outside the range of the reference compounds (n-alkanes).

stationary phases due to the wide possibility of modifying sorptive properties, and therefore selectivity of the obtained stationary phases.

The retention of individual groups of chemical compounds confirms the above assumptions: high retention compared to saturated hydrocarbons of low polarity is exhibited by polar compounds:

– alcohols having specific interactions through the hydroxyl group. In this case, interactions can take place with protonated L-proline as well as with xylitol;
– pyridine and its alkyl derivatives are capable of proton-acceptor interactions. In this case, interactions with the carboxyl group of L-proline, with the hydrogen atom of protonated L-proline and with the hydroxyl groups of xylitol should be expected. At the same time, the synthesized DES provides a good separation of analytes within each group.

The investigated stationary phase also exhibits a significant selectivity in terms of occurrence of:

– alkyl substituents – compounds without alkyl groups have stronger interactions with DES (values of Δt_r) than their alkylated derivatives. This effect is observed, among others, for aromatic hydrocarbons (benzene vs alkyl derivatives), thiophene and its alkyl derivatives as well as pyridine and its derivatives;
– aromatic ring – increased retention for compounds with a phenyl group in their structure. This effect is noticeable for thiophenol (compared to aliphatic thiols) as well as for nitrobenzene and 4-methylbenzaldehyde.

The second of the parameters used, the interaction coefficient (I_p), allows to additionally assess the nature of the stationary phase. In this case, the retention of the compound on the investigated stationary phase is compared to the expected value (calculated with respect to *n*-alkanes), but the molar mass is used as a physicochemical parameter for the calculations. Similarly to the Δt_r values, high I_p values were observed for alcohols and pyridines, and relatively high for ketones.

The fact that several different types of sorptive interactions may take place makes the developed stationary phase useful for solving specific resolution problems in which there is a co-elution of analytes when using commercial stationary phases. On the other hand, high retention of two groups of chemical compounds makes the developed stationary phase useful as the so-called sorption trap in multicolumn separation procedures.

3.3. Comparison of selectivity of DES phase with commercial GC stationary phases

The selectivity of the synthesized DES-based phase with commercially available stationary phases was compared by following a standard protocol: the Rohrschneider-McReynolds constants were determined [28–31]. This approach provides the identification of the main types of sorptive interactions occurring for the investigated stationary phase and a comparison of their effect on the retention of five test substances relative to the stationary phases available. The comparison is based on the differences in retention index values for the test substances (benzene, n-butanol, 2-pentanone, nitropropane, pyridine) on the investigated stationary phase and on squalane (considered to be the most nonpolar stationary phase). McReynolds constants calculated for the DES-based stationary phase are listed in Table 2. These values were compared with those for commercial GC stationary phases and with the first DES-based stationary phase developed previously [25,31]. The test compounds used represent various specific interactions with the stationary phase (Fig. 4).

Calculations carried out for the investigated DES-based phase showed a significant value of the $\Sigma(\Delta I)$ – it is equal on average (for two columns) to 1717, *i.e.* the stationary phase is polar [32]. The components of the ΔI values confirm the selectivity of DES as described in the previous paragraph. However, a comparison of individual components of the test compounds reveals that the DES-based phase is characterized by an unprecedented selectivity, not matched by any of the GC stationary phases available on the market.

An inspection of the differences of retention index values with respect to squalane (ΔI) for individual test substances revealed that the synthesized DES-based phase has the strongest interactions with pyridine ($\Delta I = 661$), unprecedented for commercially available stationary phases, followed by strong interactions with nitropropane and 1-butanol (ΔI values equal to 461 and 417, respectively). At the same time, according to the McReynolds constants, the investigated phase does not exhibit selectivity for aromatic compounds relative to squalane ($\Delta I = 0$). Evidently, the selectivity of the developed stationary phase is different from that of the commercially available stationary phases [26] and the previously developed stationary phase based on DES. Furthermore, presence of L-proline makes some potential for application of this stationary phase for chiral separations. This aspects will be further studied in future papers about DES-based stationary phases for GC.

3.4. Retention stability, analysis-to-analysis and column-to-column repeatability

As mentioned earlier, the stationary phase for GC must ensure the repeatability of retention of the separated chemical compounds during the repeated use of the same column (analysis-to-analysis repeatability, $R_{a\text{-}a}$). At the same time, commercialization of the phase requires the development of reproducible conditions for the production of the stationary phase and ultimately the chromatographic columns (column-to-column repeatability, $R_{c\text{-}c}$).

For the stationary phase developed, both aspects of application to routine analyses were investigated. In the first case ($R_{a\text{-}a}$) by checking the stability of retention characteristics for successive analysis cycles in the temperature program, and in the second ($R_{c\text{-}c}$) by comparing the retention parameters of two columns made in the same way using independently synthesized batches of DES.

3.4.1. Analysis-to-analysis repeatability

The analysis-to-analysis repeatability $R_{a\text{-}a}$ was typical for gas chromatography. Samples were injected using an autosampler with a standard injection rate. The spread of retention times of test analytes for three consecutive injections did not exceed 0.01 min.

3.4.2. Column-to-column repeatability

The advantage of using DESs as sorption media is the simplicity of their synthesis – in most cases heating two compounds with simultaneous mixing is sufficient. In the case of the DES used, in the first step a homogeneous aqueous solution of the two components with the addition of hydrochloric acid is obtained, from which water is distilled off by means of an automated rotary evaporator. The next steps, i.e. immobilization and packing of the column are also standardized, hence no differences in the properties of the columns obtained were anticipated.

The test results for both columns compiled in Table 2 as a comparison of McReynolds constants revealed that the differences in McReynolds constants values are insignificant, which demonstrates the good repeatability of the properties of independently prepared columns.

3.4.3. Temperature stability

Temperature stability of the DES-based stationary phase was assessed by comparing the stability of retention times of the test compounds after 50 chromatographic runs using the temperature program described in Experimental but changing the final column temperature. Typically, thermal stability of material could be characterized by thermogravimetric analysis (TGA). However, in our opinion TGA due to its simplicity and robust methodology causes less sensitivity for evaluation of thermal stability of stationary

Table 2
Comparison of McReynolds constants values for developed DES phase and commercial stationary phases for GC [32].

Phase	Benzene x' ΔI	1-Butanol y'	2-Pentanone z'	Nitropropane u'	Pyridine s'	Sum Σ(ΔI)
DES Phase L-proline-Xylitol	**0**	**417**	**177**	**461**	**661**	**1716**
DES Phase L-proline-Xylitol (repeated)	**0**	**416**	**178**	**462**	**662**	**1718**
Phase DES TBAC-n-C_{16}COOH	57	395	147	278	298	1174
OV-1	16	55	44	65	42	222
Dexsil 400 carborane/methylphenyl silicone	72	108	118	166	123	587
SPB-20	67	116	117	174	131	605
DC702	77	124	126	189	142	658
OV-1701	67	170	153	228	171	789
SPB-1701	67	170	153	228	171	789
Dexsil 410 carborane/methylcyanoethyl silicone	72	286	**174**	249	171	952
SPB-50	125	175	183	268	220	971
Span 80	97	266	170	216	268	1017
Castorwax	108	265	**175**	229	246	1023
Atpet 200	41	282	186	235	289	1033
Triton X-200	117	289	172	266	237	1081
Polypropylene glycol	128	294	173	264	226	1085
Pluracol P-2010	129	295	**174**	266	227	1091
Atper 200	108	282	186	235	289	1100
UCON LB 1715	132	297	180	275	235	1119
Dibutoxyethyl adipate	137	278	198	300	235	1148
OV-25	178	204	208	305	280	1175
Diethoxyethyl sebacate	151	306	211	320	274	1262
Dibutoxyethyl phthalate	151	282	227	338	267	1265
SP-1220	207	297	153	283	328	1268
DC QF-1 (FS 1265)	144	233	355	**463**	305	1500
Cresyldiphenylphosphate	199	351	285	413	336	1584
OV-330 silicone - Carbowax	222	391	273	417	368	1671
Diethoxyethyl phthalate	214	375	305	446	364	**1704**
Carbowax 20M	322	536	368	572	510	2308

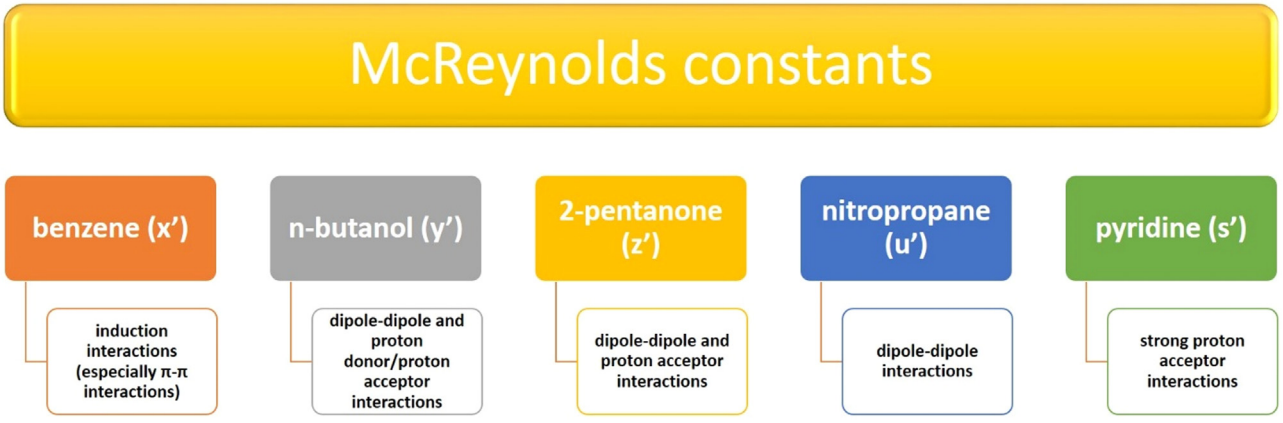

Fig. 4. Types of interactions with stationary phase characterized by McReynolds constants.

phase, comparing to systematic analysis of data acquired by gas chromatograph with FID detector. In case of GC-FID it is possible to inspect even very slight decomposition of DES by FID having sensitivity below 1 ppm (TGA present the data in approx. 1% changes of weight). Secondly, it is possible to evaluate qualitatively the effect of temperature by retention and selectivity changes. In case of TGA only weight loose would be monitored (reactions of polymerization or transformation of material will be not easily to detected). Thus GC technique allows to directly determine temperature limit of specific material for GC Separations.

The comparison was performed using five representative test compounds, which are used for the determination of McReynolds constants values. The stability was examined for the programmed final oven temperature up to 170°C. The studies revealed complete stability of the DES-based phase after 50 chromatographic runs to a final oven temperature of 140°C. A decrease in retention amounting to 3.1-5.5% and 6.9-11.0% was observed after chromatographic runs ending at 150°C and 160°C, respectively. A significant deterioration of sorptive properties of the DES phase was observed for a final oven temperature of 170°C.

The retention data obtained demonstrate the suitability of the developed DES phase to separation of VOCs. During all studies carried out with the prepared columns, each of the columns was operated for at least a week (with breaks), maintaining its original properties, which demonstrates long-term stability of the DES-based stationary phase.

4. Conclusions

The paper presents a new stationary phase for gas chromatography based on the deep eutectic solvent prepared from L-proline protonated with hydrochloric acid and xylitol in a 5:1 mole ratio. The studies revealed that in the case of using protonated L-proline, from among three acids tested: hydrochloric, phosphoric and sulfuric, only HBA obtained with hydrochloric acid ensures obtaining a phase characterized by good peak symmetry and efficiency of GC columns. The developed DES provides an interesting selectivity towards VOCs – the stationary phase is polar, but the values of McReynolds constants are very diverse – such selectivity is not common for commercially available GC columns. The columns prepared are characterized by good efficiency and long-term stability.

In case of environmental analysis, often a complex mixture of volatile organic compounds has to be separated. Many separation issues related to co-elution of analytes would be solved by application of new types of stationary phases having specific selectivity. DES-based stationary phases have a potential to be a one of first-choice solutions. Secondly, popularity of two dimensional separations makes such phases very attractive for orthogonal separation systems. It would easily differentiate volatiles by their polarity.

Development of stationary phases for GC based on DESs is also a step forward in green analytical chemistry. Typically used stationary phases are manufactured by several steps, including chemical synthesis of specific precursors and (in most of the cases) controlled polymerization, including crosslinking. In terms of rules of green chemistry, application instead of such approaches a compounds of natural origin followed by simple mixing of components assisted by middle heating seems to be a significant improvement.

Declaration of Competing Interest

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

CRediT authorship contribution statement

Malwina Momotko: Investigation, Conceptualization, Methodology, Formal analysis, Writing – original draft, Validation, Data curation, Writing – review & editing. **Justyna Łuczak:** Supervision, Writing – review & editing. **Andrzej Przyjazny:** Writing – review & editing. **Grzegorz Boczkaj:** Conceptualization, Methodology, Validation, Writing – review & editing, Supervision, Project administration, Funding acquisition.

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