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# First deep eutectic solvent-based (DES) stationary phase for gas chromatography and future perspectives for DES application in separation techniques

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ABSTRACT

The paper presents the first application of deep eutectic solvents (DES) as stationary phases for gas chromatography. DES obtained by mixing tetrabutylammonium chloride (TBAC) as a hydrogen bond acceptor (HBA) with heptadecanoic acid being a hydrogen bond donor (HBD) in a mole ratio of HBA:HBD equal to 1:2 was characterized by its ability to separate volatile organic compounds (VOCs). The Rohrschneider – McReynolds constants determined reveal that the synthesized DES is a stationary phase of medium polarity. A detailed retention characteristic was determined for a number of groups of chemical compounds, including aromatic hydrocarbons, alcohols, ketones, sulfides and thiophene derivatives. The synthesized DES was found to have a high selectivity towards alcohols. At the same time, the investigated stationary phase was found to have specific interactions with some analytes. For example, a stronger retention was observed for 1-hexanol and 1-heptanol compared to other alcohols. Retention times of these two alcohols are longer by 191% and 300%, respectively, relative to the expected value based on their boiling point. Such an increased retention is caused by a synergistic effect of various kinds of interactions – the possibility of formation of hydrogen bonds between the DES and the hydroxyl group of alcohols and hydrophobic interactions of alkyl chains of the DES with the alkyl chain of alcohols. The ability to modify properties of DESs by replacement of HBA or HBD with a different chemical compound or by dissolving in DES macromolecular substances makes the proposed stationary phase highly flexible. In addition to using the developed DES in chromatographic techniques, the retention data collected indicate the possibility of its application to other separation techniques, i.e. extractive distillation.



## 1. Introduction

Deep eutectic solvents (DES) are mixtures of chemical compounds having a substantially lower melting point compared to that of pure components. The possibility of obtaining DESs that remain liquid under standard conditions and often at temperatures substantially below 0 °C opens up new venues for research on novel DESs, for characterization of their physico-chemical properties and their practical applications [1-4].

Many DES components are inexpensive and the products exhibit unique interactions with a number of groups of chemical compounds. Consequently, deep eutectic solvents have largely become an object of interest of separation scientists – as extractants [5,6].

At the same time, one of the hot topics in separation techniques is the development of novel sorption media for the operations in the gas-solid and gas-liquid systems, including stationary phases for gas chromatography [7-10].

No papers on application of DESs as stationary phases for gas chromatography have been published thus far [11]. The use of DES as stationary phases in chromatography should result in preparation of novel sorption media having an unprecedented selectivity. The use of chemical compounds for DES synthesis that have not been used for this purpose so far can solve a number of resolution problems and can lead to the development of new conditions for the separation of complex mixtures for which the existing separation procedures are tedious and time-consuming or are based on expensive and often toxic chemicals.

The paper presents the study of the first GC stationary phase based on DES composed of tetrabutylammonium chloride-heptadecanoic acid (TBAC/$C_{16}$-$CO_2H$). The obtained DES was immobilized on a chromatographic support (Chromosorb W-AW/DMCS), and the stationary phase prepared in this way was packed into a GC column. The investigations were carried out using gas chromatography with flame ionization detection (GC-FID) for selected groups of VOCs. To examine the selectiv-

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ity of the stationary phase studied in terms of its potential applicability, retention parameters were determined for the investigated analytes.

## 2. Experimental

### *2.1. Materials*

Tetrabutylammonium chloride and heptadecanoic acid with a purity >99% (Sigma Aldrich) were used in the investigations. In order to prepare the stationary phase, DES was immobilized on a support: Chromosorb W AW-DMCS (80/100mesh) from acetone (analytical reagent, POCH). Packed columns were prepared from thin-walled steel tubing (1/8" ID, L= 2.70 m). Both ends of the column were plugged with silanized glass wool (Supelco).

Standards of VOCs (Sigma Aldrich) and a mixture of *n*-alkane standards ranging from *n*-$C_5$ to *n*-$C_{17}$ (Analytical Controls) were used in the investigations. In both cases, carbon disulfide (analytical reagent, Merck) was used as a solvent for the analytes. The following gases were used in the GC analysis: carrier gas was nitrogen (N5.0, Linde Gas), and the FID detector gases were air (N5.0, Linde Gas) and hydrogen (N5.5, from a hydrogen generator).

### *2.2. Apparatus*

Chemicals were weighed on a AS.310.R2 analytical balance (Radwag). DES was prepared using a 06-MSH-PRO-T magnetic stirrer (Chemland). Immobilization of DES on the support was carried out using Rotavapor R-300 rotary evaporator (Buchi). Packing columns was aided by a CRVpro4 vacuum pump (Welch). Characterization of retention properties of the DES-based stationary phase was performed using a Clarus 500 gas chromatograph (Perkin Elmer, USA) equipped with an autosampler, a split/splitless injection port and a flame ionization detector (FID). A PGX-H2 500 hydrogen generator (Perkin Elmer) was connected to the chromatograph.

### *2.3. Synthesis of deep eutectic solvent*

Deep eutectic solvent was obtained by mixing weighed amounts of tetrabutylammonium chloride and heptadecanoic acid. The two components were weighed on an analytical balance and placed in a vial equipped with a magnetic stirring bar. The closed vial was placed in a beaker filled with water and the mixture was stirred using a magnetic stirrer at 50°C until a homogeneous liquid phase was obtained. Two independent syntheses were carried out for the selected composition of DES in order to determine reproducibility of properties of the synthesized stationary phases. Melting point of the DES was determined by heating the mixture from room temperature by 1°C and visual inspection of the state of the sample.

### *2.4. Immobilization of DES on solid support and preparation of packed columns for gas chromatography*

Subsized particles were removed from commercial Chromosorb W-AW-DMCS 80-100 mesh (Johns-Manville) by decantation from methanol. Next, the support was activated in a vacuum oven at 230 °C for 4 hours. The activated support was left in the evacuated oven to cool to about 30 °C and after taking it out of the oven it was instantly poured into a DES solution in acetone (1 g DES in 150 mL of acetone). The suspension was thoroughly mixed in the flask and then the solvent was evaporated using a rotary evaporator. Thus prepared stationary phase was used to pack GC columns using the dry pack method . Next, the column was conditioned in the flow of inert gas (nitrogen, 40 mL/min) for 1 hour at 30 °C, followed by a temperature ramp from 30 °C to 100 °C at 1 °C/min. Afterward, the column outlet was connected to the FID and the column was heated at 100 °C until the chromatographic baseline stabilized.

### *2.5. Investigation of retention characteristics of stationary phase by gas chromatography*

Solutions of standard mixtures at concentrations *ca.* 500 ppm were prepared either in carbon disulfide or in methanol depending on solubility of individual analytes. Dead time was determined by injection of 0.1 mL of a mixture of methane in nitrogen (10 ppm) using a gastight syringe.

Splitless injection was used in all determinations and the injection volume of standard solutions was 1 μL. Linear velocity of the carrier gas was 4.21 cm/s. Temperatures of both the injection port and FID were equal to 300 °C. Chromatographic analyses were carried out using a temperature program. The initial oven temperature was 40 °C (held for 1 min), followed by a ramp to the final temperature (100 °C) with the rate of increase of 10 °C/min. The final oven temperature was held for 34 min.

Retention times of the standards were determined from the chromatograms recorded. On their basis, retention (**k**) and selectivity (**α**) factors as well as the number of theoretical plates (**N**) were computed. The retention times obtained ($\mathbf{t_{r,real}}$) were then compared with the expected retention times for a nonpolar stationary phase – predicted from the boiling point (**bp)** of the analytes ($\mathbf{t_{r,bp}}$). The expected retention times were computed from the experimentally determined relationship of retention times on boiling points for reference compounds – *n*-alkanes $\mathbf{t_{r,bp} = f(bp)}$. This approach, assuming the linear dependence of retention time on boiling point of the separated compounds forms the basis for simulated distillation [12, 13]. On this basis, the relative percent deviation of retention time $\Delta t_r\%$ was also calculated. In addition, interaction coefficients ($\mathbf{I_p}$) were calculated using a modified Davis method [14]. The $I_p$ value was calculated as the difference between $\mathbf{t_{r,real}}$ and the theoretical value for a given analyte determined from the calibration curve of dependence of common logarithm of retention times of *n*-alkane standards on their molar mass (1).

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

where:

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

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]

### *2.6. Investigation of analysis-to-analysis and column-to-column repeatability of retention parameters*

Repeatability of retention times was estimated on the basis of three analyses of the same mixture using a given column (analysis-to-analysis). The results obtained were compared with the data obtained after 50 chromatographic runs in order to evaluate the stability of the stationary phase with the temperature program used.

Independently, chromatographic properties of columns containing two batches of DES synthesized under identical conditions were compared in order to determine column-to-column repeatability. The repeatability of properties of the stationary phase was evaluated on the basis of the retention factor and McReynolds constants.

## 3. Results and discussion

Due to a variety of possible combinations of hydrogen bond acceptor (HBA) – hydrogen bond donor (HBD), deep eutectic solvents have a

tremendous potential applicability to separation techniques. However, no papers regarding the application of DESs in gas chromatography have been published so far. The possibility of obtaining a liquid state at ambient temperature for chemical compounds with high melting points present in DESs brought about a chance for the development of novel stationary phases offering unprecedented sorption properties. Over the last dozen or so years, similar interest accompanied the research on stationary phases for GC based on ionic liquids [15,16].

The development of new applications for DESs as more environmentally friendly solvents than traditional organic solvents or absorbents has been obvious from the beginning and has not presented many problems. However, the use of DESs as stationary phases for GC requires much more rigorous physicochemical characteristics of their components. Firstly, the compounds making up a DES must have a negligible volatility and high thermal stability at the expected operating temperature of the stationary phase. The DES used as a stationary phase must have a long-term stability of sorption properties ensuring reproducibility of retention of separated analytes after many separation cycles. The first requirement regarding low volatility of DES components eliminates a number of DESs synthesized thus far, especially the hydrophobic ones, based on camphor or thymol, but also the hydrophilic ones based on phenol or low-boiling alcohols. In addition, many compounds exhibiting interesting sorption interactions in DESs used as solvents, *e.g.* those based on amino acids, is not sufficiently stable thermally (decomposition temperature below 100 °C).

Two chemical compounds meeting the above requirements were selected for this research. TBAC (melting point 70 °C) was used as a HBA while heptadecanoic acid (melting point 61.3 °C) constituted a HBD – in a mole ratio of HBA:HBD - 1:2. The structures of DES components are presented on figure 1. This combination resulted in formation of a clear colorless eutectic solvent with a melting point of 28 °C.

To evaluate applicability of the synthesized DES as a stationary phase for GC, a traditional method of column packing was used. Chromatographic parameters of the stationary phase obtained were determined on the basis of separation of straight chain saturated hydrocarbons (*n*-alkanes) and of a number of groups of VOCs.

Retention characteristics of the stationary phase obtained were determined using a total of 40 test compounds. The retention factor, relative percent deviation of retention time and the interaction coefficient were calculated for each of the test compounds.

### *3.1. Characteristic of columns with DES stationary phase*

Standard packed GC columns were used in this research. This allowed to use the simplest, well tested approach ensuring preliminary evaluation of the potential of DESs as stationary phases for GC. In case of capillary columns, the complexity of coating procedure and a number of details of preparation strongly affect the final column efficiency and symmetry of chromatographic peaks. An assumption was made that in the case of positive results for packed columns, further investigations of novel DESs suitable for GC and optimization of conditions of preparation of capillary columns will be continued. The following column dimensions were used in this work: length 2.7m and diameter 1/8". The synthesized DES was fully soluble in acetone which assured optimal conditions for static coating of the DES on the support during evaporation of the solvent by means of a rotary evaporator. A typical loading (10% w/w) of the support Chromosorb W(AW-DMCS) with the stationary phase was used. The column packing was free-flowing; no clumping of the packing was observed. No differences were observed during DES coating onto the support and packing of the column compared to the handling of typical liquid stationary phases for GC.

The efficiency of the packed columns was 11500 and 7560 theoretical plates for ethylbenzene and propylbenzene, respectively and 10150 and 7960 for *n*-nonane and *n*-decane, respectively, as the test compounds. On the average, the column efficiency was 3440 theoretical plates per meter, which is a good result for packed columns.

An example of separation of a mixture of aromatic hydrocarbons in shown in Figure 2. Fundamental physicochemical properties and retention parameters of compounds separated using the stationary phase based on DES are listed in Table 1. The retention factors as well as specific parameters describing the selectivity of the phase were calculated.

The investigations revealed that the synthesized DES used as a stationary phase for GC is characterized by a surprising selectivity with respect to the groups of analytes tested. From the point of view of DES structure (four butyl groups in HBA and a long 17-carbon chain in HBD), a highly hydrophobic stationary phase was to be expected. Assuming as the main retention criterion for analytes separated on stationary phases of low polarity their boiling point and the fundamental principle used in partition gas chromatography – like dissolves like, it was expected that more polar analytes would have a low retention and selectivity compared to *n*-alkanes. However, the results of this study reveal a completely different retention characteristic of the investigated analytes. The DES used has a centrally located structure of high polarity – a positively charged quaternary nitrogen atom that interacts with a chloride anion counterion that forms a hydrogen bond with the carboxylic acid hydroxyl group. The observed retention of individual groups of VOCs indicates strong interactions of the stationary phase with the analytes containing polar functional groups. This is especially true of alcohols, ketones and the nitrogen-containing compounds.

The retention characteristic of the synthesized phase is not typical. A satisfactory separation of the analytes from the groups studied and an interesting selectivity were observed. A comparison of the DES selectivity towards individual groups of the analytes demonstrates that the phase is characterized by a very high selectivity towards alcohols. This surprisingly increased retention of alcohols can result from the interactions of alkyl chains of the DES with alkyl chains of the analytes but also from the interactions of the hydroxyl group of alcohols with TBAC, which is a hydrogen bond acceptor in the DES, or with the carboxyl group of HBD. Consequently, the synthesized stationary phase can provide a variety of interactions with the analytes which results in a unique selctivity which might be useful in solving particular separation problems. This is an essential feature which can be helpful if the separation of a key pair of analytes from the same group is needed. In addition to application of DESs in one-dimensional GC, their high selectivity to selected groups of chemical compounds can find use in multidimensional techniques, especially the heart cut option [17,18], wherein a target group of analytes undergoes selective retention in the first dimension by using an appropriate stationary phase, which allows removing the analytes from a complex matrix, followed by elution of the fraction retained to the second dimension where it is separated into individual components.

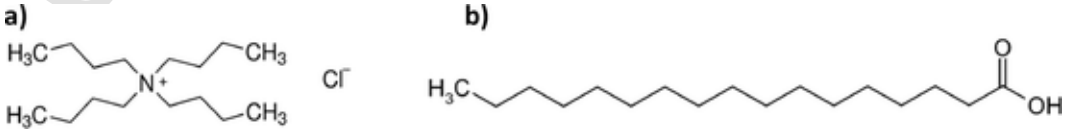


**Figure 1.** Chemical structures of a) TBAC and b) heptadecanoic acid.

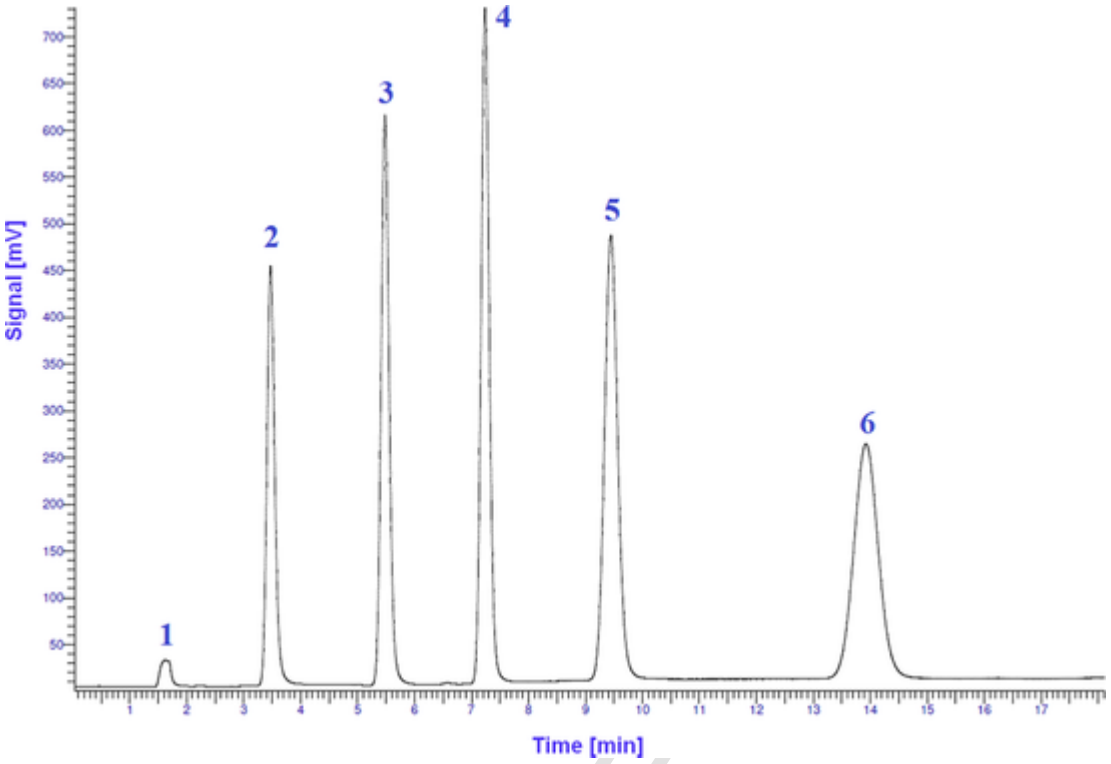


**Figure 2.** Separation of aromatic hydrocarbons (mixture containing 0.5% of each standard in carbon disulfide, chromatographic conditions as described in section 2.5). Injection volume 1 μL in splitless mode. Temperature program: 40 °C (held for 1 min), then ramped to 100 °C (held for 34 min) at 10 °C/min. 1 – carbon disulfide; 2 – benzene; 3- toluene; 4- ethylbenzene; 5- *n*-propylbenzene; 6- *n*-butylbenzene.

The investigated DES also has a specific selectivity toward some tested analytes, for which a significantly increased retention was observed. These include 1-heptanol and 1-hexanol. Thus, the examined DES could possibly be applied to cases in which the critical pair of analytes is not satisfactorily separated on commercial stationary phases.

### *3.2. Comparison of selectivity of the DES-based phase with commercially available GC stationary phases*

In order to compare the selectivity of the synthesized DES-based phase with commercially available GC stationary phases, the Rohrschneider – McReynolds constants were determined [19-22]. This approach is considered to be most appropriate for the characterization of the main properties of a stationary phase related to typical sorptive interactions between the analytes being separated and the stationary phase in GC. The approach is based on comparison of the differences in retention indices for test substances (benzene, *n*-butanol, 2-pentanone, nitropropane, pyridine) on the investigated stationary phase and on squalane. The test compounds selected for the development of this procedure are characterized by various types of sorptive interactions which can take place during their separation by GC. The differences in retention indices for test compounds allow a comparison of the developed stationary phase with commercially available stationary phases. McReynolds constants calculated for the DES-based stationary phase are compiled in Table 2. These values are compared with those for commercially available GC stationary phases [23]. The test compounds used represent various specific interactions having a dominating effect on the difference in retention between the examined phase and a nonpolar phase squalane. Benzene is characterized by induction interactions (especially π-π interactions), *n*-butanol - by dipole-dipole and proton donor/proton acceptor interactions, 2-pentanone – dipole-dipole and proton acceptor interactions, nitropropane – dipole-dipole interactions, and pyridine – strong proton acceptor interactions. The investigations revealed that the sum of the differences of the retention indices (Σ(ΔI)) of the investigated DES-based stationary phase is equal to 1174. This value places the synthesized stationary phase as being of intermediate polarity [23]. However, a comparison of individual components of the test compounds reveals that the DES-based stationary phase is characterized by a different selectivity, not offered by any of the commercially available stationary phases. An inspection of the retention index differences for the test compounds on the developed stationary phase with respect to squalane (ΔI) reveals that the DES-based phase has the strongest interactions with 1-butanol (ΔI = 395), followed by strong interactions with nitropropane and pyridine (ΔI values equal to 278 and 298, respectively). A comparison of the obtained McReynolds constants with the data available for commercial stationary phases [23] demonstrated that the selectivity of the DES-based stationary phase is different from that of the other stationary phases.

### *3.3. Investigation of selectivity of DES-based phase with respect to individual groups of chemical compounds*

In addition to characterization of the synthesized stationary phase described above, additional parameters related to selectivity of separation were determined for individual groups of VOCs. A complete separation of a specific mixture does not prove the selectivity of a stationary phase since in gas chromatography a number of mixtures can be separated based solely on differences in boiling points. This rule is valid for stationary phases of low polarity which do not offer any specific selectivity and the order of elution fully corresponds with a boiling point, which forms the basis for so-called simulated distillation. Based on the relative percent deviation of retention time obtained on the investigated stationary phase from the expected value calculated from the boiling point ($\Delta t_r$%), the extent to which the investigated stationary phase has additional, specific interactions with the groups of separated

**Table 1**
Retention data measured for developed DES stationary phase.

| Boiling point(b.p)[°C] | Molecular weight(M) [g/mol] | Compound | Retention time($t_r$)[min] | Retention factor(k)[-] | Theoretical retention time by b.p.($t_r$ theor.)[min] | Difference between measured and theoretical retention time($\Delta t_r$)[%] | Interaction coefficient($I_p$)[-] |
|---|---|---|---|---|---|---|---|
| **Aromatic hydrocarbons** | | | | | | | |
| 80.1 | 78.11 | Benzene | 3.49 | 7.3 | 2.09 | 66.64 | 51 |
| 110.6 | 92.14 | Toluene | 5.48 | 12.0 | 3.78 | 45.07 | 40 |
| 136 | 106.17 | Ethylbenzene | 7.24 | 16.2 | 5.75 | 25.98 | 26 |
| 144 | 106.17 | *o*-Xylene | 8.16 | 18.4 | 6.47 | 26.04 | 31 |
| 159 | 120.19 | Propylbenzene | 9.45 | 21.5 | 7.98 | 18.49 | 24 |
| 183 | 134.22 | Butylbenzene | 13.99 | 32.3 | 10.75 | 30.11 | 27 |
| **Alcohols** | | | | | | | |
| 97 | 60.06 | 1-Propanol | 6.85 | 15.3 | 2.94 | 133.39 | 106 |
| 98 | 74.12 | 2-Butanol | 6.7 | 15.0 | 2.99 | 124.61 | 87 |
| 102 | 88.15 | *t*-Amyl alcohol | 6.68 | 14.9 | 3.23 | 106.97 | 58 |
| 116 | 74.12 | 1-Butanol | 9.25 | 21.0 | 4.15 | 122.74 | 101 |
| 119 | 88.15 | 2-Pentanol | 8.7 | 19.7 | 4.37 | 99.24 | 70 |
| 138 | 88.15 | 1-Pentanol | 13.7 | 31.7 | 5.92 | 131.77 | 90 |
| 156 | 102.17 | 1-Hexanol | 22.3 | 52.1 | 7.66 | 191.09 | 79 |
| 160 | 100.16 | Cyclohexanol | 15.4 | 35.8 | 8.08 | 91.04 | 65 |
| 175 | 116.2 | 1-Heptanol | 39.3 | 92.7 | 9.78 | 302.43 | 90 |
| **Ketones** | | | | | | | |
| 92 | 86.13 | Methyl isopropyl ketone | 3.7 | 7,8 | 2.66 | 38.97 | 38 |
| 101 | 86.13 | 2-Pentanone | 4.38 | 9.4 | 3.17 | 38.28 | 45 |
| 124 | 114.19 | Diisopropyl ketone | 5.8 | 12.7 | 4.75 | 21.21 | 8 |
| 127.6 | 100.16 | 2-Hexanone | 6.41 | 14.3 | 5.04 | 27.22 | 27 |
| 131 | 84.12 | Cyclopentanone | 7.07 | 15.8 | 5.32 | 32.93 | 71 |
| 140 | 100.12 | Acetylacetone | 7.1 | 15.9 | 6.10 | 16.48 | 31 |
| 147 | 114.19 | 3-Heptanone | 8.1 | 18.3 | 6.76 | 19.82 | 23 |
| 155.6 | 98.14 | Cyclohexanone | 9.96 | 22.7 | 7.62 | 30.72 | 48 |
| 169 | 112.17 | 3-Methylcyclohexanone | 12.26 | 28.2 | 9.08 | 35.07 | 43 |
| **Thiophene and its alkylated derivatives** | | | | | | | |
| 84 | 84.14 | Thiophene | 3.99 | 8.5 | 2.27 | 75.92 | 46 |
| 112 | 98.17 | 2-Methylthiophene | 5.74 | 12.7 | 3,87 | 48.22 | 27 |
| 116 | 98.17 | 3-Methylthiophene | 6.07 | 13.5 | 4.15 | 46.16 | 29 |
| 133 | 112.19 | 2-Ethylthiophene | 7.5 | 16.9 | 5.49 | 36.68 | 22 |
| **Sulfides and disulfides** | | | | | | | |
| 91 | 90.19 | Diethyl sulfide | 3.61 | 7.6 | 2.61 | 38.29 | 27 |
| 110 | 94.2 | Dimethyl disulfide | 5.27 | 11.5 | 3.74 | 41.02 | 33 |
| 152 | 118.24 | Diethyl disulfide | 8.84 | 20.0 | 7.25 | 21.89 | 23 |
| 142 | 122.25 | Dipropyl sulfide | 7.24 | 16.2 | 6.29 | 15.15 | 10 |
| 188 | 146.29 | Dibutyl sulfide | 14.17 | 32.7 | 11.39 | 24.42 | 16 |
| 193 | 150.31 | Dipropyl disulfide | 17.93 | 41.7 | 12.05 | 48.85 | 22 |
| 200 | 178.36 | Di-*t*-butyl disulfide | 17.57 | 40.8 | 13.35 | 31.61 | -21 |
| **Other compounds** | | | | | | | |
| 115.2 | 79.1 | Pyridine | 8.5 | 19.3 | 4.10 | 108.02 | 91 |
| 202 | 123.11 | 4-methylbenzaldehyde | 29.1 | 68.3 | 13.28 | 119.09 | 69 |
| 132 | 89.09 | Nitropropane | 6.9 | 15.4 | 5.40 | 27.53 | 57 |

analytes can be estimated. The strongest interactions with the stationary phase are exhibited by alcohols (deviations ranging from 91% to 302 %). For the other investigated groups in the majority of cases the values of $\Delta t_r$ also indicate an increased retention at a level of several dozens of %. A comparison of magnitude of deviations of retention times for the investigated stationary phases is shown in Table 1.

The second of the calculated parameters – the interaction coefficient ($I_p$) allows evaluation of deviation of retention with respect to the expected value based on the molecular mass of the compound. Also in this case, *n*-alkanes are used as reference compounds. As a rule, for chemical compounds belonging to the same homologous series there is a strong correlation between molar mass and boiling point and thus also retention in GC. In contrast, there is no such relationship for compounds greatly differing in elemental composition, for example benzene ($C_6\mathbf{H}_6$, b.p. **80.1**°C, M 78.11 g/mol) and hexafluorobenzene ($C_6\mathbf{F}_6$, b.p. **80.1**°C, M 186.05 g/mol) [24].

Classification of the strength of interactions of the tested analytes with the investigated stationary phase using the interaction coefficient revealed that the strongest interactions take place for alcohols. The agreement of the results obtained for the two parameters demonstrates the existence of specific interactions of the developed stationary phase. For alcohols, the DES-based phase will exhibit specific interactions of a mostly proton acceptor type (with respect to the hydroxyl group of an alcohol).

**Table 2**
Comparison of McReynolds constants values for developed DES phase and commercial stationary phases.

| Phase | Benzene | 1-Butanol | 2-Pentanone | Nitropropane | Pyridine | Sum |
|---|---|---|---|---|---|---|
| | x' | y' | z' | u' | s' | Σ(ΔI) |
| | ΔI | | | | | |
| **DES PHASE** | **57** | 395 | 147 | 278 | 298 | **1174** |
| **DES PHASE (repeated)** | **56** | 396 | 147 | 279 | 299 | **1177** |
| 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 |

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

The use of a new sorptive medium as a stationary phase for chromatography requires confirmation that it ensures repeatability of retention parameters. This aspect is related to problems associated with maintaining repeatability of properties of material (selectivity) synthesized in consecutive batches, repeatability of column parameters, and for each of the columns preservation of retention characteristics during successive chromatographic analyses. Stability of a stationary phase in time ensures its applicability to routine analyses. In this work, the repeatability of sorptive properties of two independently prepared columns was investigated – for each of the columns a separate batch of DES was synthesized (column-to-column repeatability). The stability of retention for each column was also evaluated after 50 cycles of the temperature program used.

#### *3.4.1. Analysis-to-analysis repeatability*

The analysis-to-analysis repeatability was found to be 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 0.007 min, and the relative standard deviation values (RSD, $n = 3$) were within 0.024-0.029%.

#### *3.4.2. Column-to-column repeatability*

The column-to-column repeatability is of particular importance in terms of commercial use of the developed stationary phase. In the case of commercially available stationary phases, often one of the steps of their manufacture is polymerization as well as cross-linking [23]. Sometimes, the synthesis of a stationary phase consists of several steps that must be strictly controlled in order to ensure the repeatability of the retention characteristics of the obtained columns [8].

In the case of the developed DES-based stationary phase, synthesis is one-step and involves simple mixing at 80 °C of two pure chemical compounds. The conditions of synthesis are thus strictly controlled and preparation of the chromatographic column (immobilization of the stationary phase and column packing) ensures repeatability with proper laboratory practice. A comparison of the values of McReynolds constants for two independently prepared columns (DES phase and DES phase(repeated)) is presented in Table 2. The comparison was made for the test compounds used to determine McReynolds constants as reflecting various interactions with the stationary phase, and thus "sensitive" to possible differences in its characteristic. The results of investigations revealed good repeatability of retention for all the examined groups of chemical compounds.

#### *3.4.3. Temperature stability*

Temperature stability of the synthesized stationary phase was evaluated by comparing the stability of retention times of the test compounds after 50 chromatographic runs using the temperature program described in Experimental but varying the final column temperature.

The comparison was performed using five representative test compounds. The stability was examined for the programmed final oven temperature ranging from 80 to 160 ˚C. The studies revealed complete stability of the DES-based phase after 50 chromatographic runs to a final oven temperature of 100 ˚C. The retention for test compounds was maintained along with the efficiency of the columns. Changes in retention were observed after chromatographic runs ending at 120 ˚C (decrease in retention time from 3.6 to 6.2%) and at 140 ˚C (decrease in retention time from 6.4 to 10.9%). A substantial decrease in sorptive properties of the DES phase was observed for a final oven temperature of 160 ˚C.

Consequently, the retention times and other parameters compiled in Table 1 refer to the oven temperature program realized over a temperature range from 40 to 100 ˚C. The retention data obtained demonstrate the suitability of the developed DES phase to separation of VOCs. In total, during all studies performed on the prepared columns, each of them was operated for about 2 weeks in temperature programming mode. During brakes between each part of studies the chromatograph was few times switched off, however most of the time the column was operated under constant flow rate of the carrier gas at initial temperature of the program (i.e. 40˚C). As the column revealed to be stable, it confirms the ability of the developed DES based stationary phase for routine practice.

### *3.5. Outlook for further investigation of DES-based phases for chromatography and other possible areas of application*

This work demonstrates the potential of DES as a stationary phase for GC. The first positive results of the investigations should open the door to a number of applications of deep eutectic solvents in chromatographic techniques. In order to obtain columns with a DES stationary phase, the classical approach based on packed GC columns was used. Due to the well-developed technique of preparation of packed columns, the effect of immobilization of the stationary phase and column packing on the separation properties obtained was limited, which allowed an objective evaluation of the applicability of the DES to gas chromatography. Further studies should aim at the development of optimum conditions of preparation of capillary columns. The DES prepared for this study is not bonded nor cross-linked (as is the case for the majority of polymeric stationary phases for GC). The selection of DES immobilization in capillary columns will be based checking the efficiency of both wall-coated open-tubular (WCOT) columns and support-coated open-tubular (SCOT) columns. The fact of commercial availability of very efficient capillary columns based on ionic liquids indicates that also in the case of deep eutectic solvents the development of commercial capillary columns is possible.

The possibility of modification of sorptive properties of DESs by replacement of an HBA or HBD allows preparation of tailored stationary phases for solving specific separation problems. At the same time, the ability of DESs to dissolve macromolecular compounds as well as ionic compounds further extends the possibility of synthesis of stationary phases having unprecedented selectivity.

The DES phase obtained in this work has a good selectivity, and in some cases even specificity, with respect to individual compounds belonging to the same group of chemicals. This property can be successfully used not only for preparative and process separation of mixtures using gas chromatography, but also in other separation techniques – for example in extractive distillation. It follows from the retention data presented in this paper that the introduction of DES to the extractive system will substantially differentiate a relative volatility for selected pairs of compounds to be separated.

## 4. Conclusions

The paper presents for the first time the applicability of a deep eutectic solvent as a stationary phase for gas chromatography. The stationary phase consisted of a mixture of tetrabutylammonium chloride as an HBA and heptadecanoic acid as an HBD – in a mole ratio HBA:HBD - 1:2 and its suitability for the separation of volatile organic compounds was demonstrated. A comparison of the McReynolds constants for the DES phase with the literature data for commercial stationary phases revealed that the DES-based phase has a dissimilar selectivity. The sum of McReynolds constants equal to 1174 positions the synthesized DES as a stationary phase of medium polarity. However, the developed DES phase exhibits its strongest interactions with alcohols which is due to the possibility of formation of hydrogen bonds between the DES components and the hydroxyl group of alcohols while at the same time retention increases substantially with the length of a carbon chain. The synergistic effect of simultaneous occurrence of different types of sorptive interactions results in an increased retention of some chemical compounds, *i.e.* 1–hexanol and 1-heptanol in alcohols. As a result, the retention times of these compounds are longer by several hundred percent compared to the expected values calculated on the basis of their boiling points.

The results of this work extend the applicability of DESs by a new group of uses – as sorptive media in chromatographic techniques. The possibility of modification of DES properties by changing one of its components or by adding an additional solute dissolved in DES create a great opportunity for the development of novel stationary phases.

## Authors statement

Malwina Momotko: Investigation, Conceptualization, Methodology, Formal analysis, Writing - Original Draft, Validation, Data Curation.

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## 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.

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