HACETTEPE JOURNAL OF BIOLOGY AND CHEMISTRY Research Article
Hacettepe J. Biol. & Chem., 2008, 36 (4), 353-362

Phenolics Separation by Molecularly Imprinted Monolithic CEC Column

Arzu Ersöz

Department of Chemistry, Anadolu University, Eskişehir, Turkey

Abstract

Capillary electrochromatography (CEC) has investigated as a technique for the separation of the following phenolic compounds: phenol, o- and m-cresol, p-dichlorophenol and 2,6-dichlorophenol, p- and o-nitrophenol. We have proposed novel CEC monolithic column for selective separation of phenolics by Methacrylamidoantipyrine, MAAP, as a new charge-transfer monomer via multiple interactions (both hydrogen bonding and hydrophobic interactions) and 4-nitrophenol. We have combined molecular imprinting with the ability of methacryloyl based modification in CEC column between p-nitrophenol and MAAP to create CEC-monolith suitable for phenol, o- and m-cresol, p-dichlorophenol and 2,6-dichlorophenol, p- and o-nitrophenol separation.

Key Words: Phenol, molecular imprinting, CEC, monolithic column

INTRODUCTION

Phenolic compounds have been interested by different researchers because of their physiological and physical-chemical properties as well as their anticarcinogenic and high antioxidant capacity. Phenols occur in wastewater of a number of industries such as high temperature coal conversion, petroleum refining, resin and plastics. Although they have anticarcinogenic and high antioxidant capacity, such aromatic compounds can be toxic when present in elevated levels and known or suspected to be carcinogens. Because of their carcinogenic properties there is a great need in wastewater for new and selective technique for phenol separation.

* Correspondence to: Arzu Ersöz
Anadolu University, Department of Chemistry, Eskişehir-Turkey

Tel: +90222 335 0580 Fax: +90222 320 4910
E-mail: arzuersoz@anadolu.edu.tr

There are different kinds of methods for the determination of phenolic compounds such as microbial degradation, adsorption on activated carbon [1], chemical oxidation using agents such as ozone, hydrogen peroxide or chlorine dioxide, incineration methods, liquid-liquid extraction, liquid extraction [2], solid-phase extraction and irradiation. One promising technique in this area is molecular imprinting. Molecular imprinting is a technique in which specific recognition sites are formed in a polymer matrix by synthesis in the presence of a template ion or molecule. Polymerization begins usually by activation of a free radical initiator with UV radiation or thermally. After polymerization is completed, the template is removed either by hydrolysis or by extraction with an appropriate solvent, leaving cavities or imprints in the polymer that are complementary in shape and functionality to the template species [3-6].

Chromatographic methods like thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC) are more sensitive and can allow the determination of a mixture of different phenolic compounds [7-10]. Solid-phase extraction (SPE) is performed for sample preconcentration and cleaning stage in these type of techniques. More recently capillary electrophoresis (CE) [11-15] has been shown to be a fast, powerful, clean and efficient separation technique for a wide variety of these compounds. Capillary electrochromatography (CEC) [16] represents a hybrid method of CE and liquid chromatography (LC). Thus, CEC combines the high efficiency of capillary electromigration with the selectivity of chromatography and is considered to combine the advantage of the high separation efficiency of CE and high selectivity offered by HPLC. So, CEC based molecularly imprinted polymers (MIPs) have shown higher efficiency than HPLC based MIPs. Up to date, the utility of the MIP-based technology in CEC is stil limited and there are three different MIPs formats for CEC: (1) the particle, (2) the coating and (3) the monolith [17].

Selective recognition of templates by MIPs is critically dependent upon the nature of binding interaction operating in the system. The kinetics and thermodynamics of template molecule recognition and rebinding by imprinted polymers depend on the nature of the interaction involved, in addition to the physical and chemical nature of the material (viz. flexibility, accessibility of the binding sites, materials shapes etc.) [18].

Different molecular interactions offer varying levels of specificity and reversibility. Thus covalent binding is highly specific and directional, yet it is notoriously slow in rebinding kinetics. On the other hand, hydrophobic interactions can be applicable to a vast array of compounds and thus is less specific, although extremely rapid. While electrostatic and hydrogen bonding interactions perform poorly in aqueous media, metal coordination interactions is not affected by the solvent environment [19]. A new approach is taken from regions containing both hydrophobic interactions and hydrogen bonding [20].

In this study, we have proposed a novel MIP monolith for CEC column for separation of phenolics. Methacrylamidoantipyrine, MAAP, was used as a new monomer via multiple interactions (both hydrogen bonding and hydrophobic interactions) and 4-nitrophenol templates. We have combined molecular imprinting with the ability of methacryloyl based modification in CEC column between p-nitrophenol and MAAP to create CEC-monolith suitable for phenol, o- and m-cresol, p-dichlorophenol and 2,6-dichlorophenol, p- and o-nitrophenol separation.

EXPERIMENTAL

Chemicals

Methacryloylchloride and aminoantipyrine were supplied by Aldrich and used as received. Ethyleneglycoldimethacrylate (EDMA) was obtained from Fluka A.G. (Buchs, Switzerland), distilled under reduced pressure in the presence of hydroquinone inhibitor and stored at 4°C until use. Azobisisobutyronitrile (AIBN) was also obtained from Fluka (Switzerland). All other chemicals were of analytical grade and were purchased from Merck AG (Darmstadt, Germany). All water used in the experiments was purified using a Barnstead (Dubuque, IA) ROpure LP reverse osmosis unit with a high flow cellulose acetate membrane (Barnstead D2731) followed by a Barnstead D3804 NANO pure organic/colloid removal and ion exchange packed-bed system.

Synthesis and Characterization of MAAP

The following experimental precedure was applied for the synthesis of MAAP [20]: 4-aminoantipyrine (0.5 g; 2.463 mmol) and pyridine (0.2 ml; 2.46 mmol) were dissolved in 100 ml of dry $CHCl_3$ and the solution was cooled to 0°C. Then, methacryloyl chloride (0.26 ml; 2.46 mmol) was poured slowly into this solution stirring magnetically at room temperature for 2 h. At the end of this chemical reaction period, the solution was washed with 50 ml of dilute HCl and 50 ml of dilute NaOH. Then, the organic phase was evaporated in a rotary evaporator and the residue was crystalized in petroleum benzene-ethylacetate.

FTIR spectra of MAAP is given below: FT-IR (KBr, cm^{-1}): 1667 cm^{-1} (amide carbonyl band), 3444 cm^{-1} (N-H band), 3528 cm^{-1} (N-H band); ^{1}H-NMR ($CHCl_3$): 2.05 ppm 3H singlet (-C=C-CH_3, vinyl methyl), 3.0 ppm; 3H singlet (-C- CH_3), 3.35 ppm 3H singlet (-N- CH_3), 5.5 ppm 1H singlet (-CH_a=C-), 5.8 ppm 1H singlet (-CH_b=C-), 7.25-8.80 ppm 4H multiplet (aromatic, $CDCl_3$ peak is also observed at 7.3 ppm with aromatic peaks), 8.80 ppm 1H singlet (aromatic), 9.1 ppm 1H singlet (N-H).

Capillary Electrochromatography (CEC) Experiments

CEC experiments were carried out with Prince CEC 760 model 3D capillary electrochromatography system, equipped with a diode-array detector. Uncoated fused-silica capillary (75 mm ID) with total length of 35.5 cm (29 cm to detector) was used for the CEC-based MIPs separations. The capillary was conditioned before use with 0.2 M NaOH and rinsed with a buffer for 20 min. Inlet buffer and analyte injection was performed for 0.2 min. at 100 mbar and 0.1 min. at 20 mbar, respectively. The detection wavelength was 210 nm and phosphate-borate buffer with pH 7.0 was selected for optimising experiments. The column temperature was maintained at 25°C.

Automated capillary rinsing, sample introduction and execution of the electrophoretic runs were controlled by a personal computer. Data processing was carried out with a commercial CE software. A washing step of 20 min with NaOH and buffer between runs was applied. Three replicate runs were performed for all conditions. The samples were introduced into the capillary at the anodic end by the application of pressure (5 bar x 3 kV).

Preparation of CEC- based MIP Monolith Columns

A fused silica capillary (75 µm i.d.) was flushed with NaOH solution followed by water and then the capillary was filled with a solution [(methacryloxy) propyl-trimethoxysilane] in acetic acid as described by Liu et al. [17] in order to introduce methacryloyl groups onto innersurface of capillary. The capillary was cut to 35.5 cm long pieces. A preorganization mixture containing 47.0 mg 4-nitrophenol, 0.68 g MAAP, 44.0 mg AIBN and 1.9 ml EDMA in 4.0 ml of acetonitrile/toluene was prepared. Then, the mixture was sonicated, introduced into the capillary and its ends were sealed by plastic rubber. The capillary column was illuminated by a UV lamp (300 W) for 1 h to perform the polymerisation reaction. A detection window was then created at the end of the monolithic polymer bed. After polymerisation, the capillary was flushed with acetonitrile-acetic acid (pH: 3.5) solution in order to remove imprinted 4-nitrophenol molecules. After flushing monolithic column with phosphate-borate buffer under pressure, a steady current was achieved within 10 min. of the application of an electric field. Acetone was used as the void marker. Capacity factor (k'), separation factor (α), the resolution (R_S) and theoretical plate numbers (N) were calculated.

RESULTS AND DISCUSSION

Formation of the 4-Nitrophenol Recognition Sites in Imprinted Polymeric Monolith in Capillary

The solution taking from the packed column after the washing with acetic acid-acetonitrile solution was investigated using CEC and 4-nitrophenol peaks were observed. This shows us, 4-nitrophenol groups have removed from the polymer leaving similar structure and shape with 4-nitrophenol. So, the capillary column having cavities which are selective to 4-nitrophenol was prepared and connected to CEC system for the separation of phenolics. The polymerization process taking place in the column and formation of selective cavities are given in Figure 1.

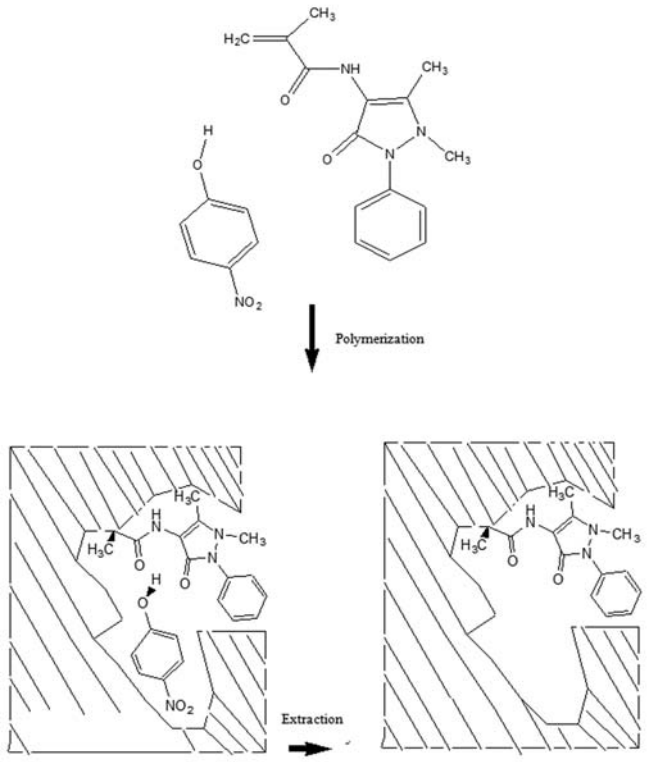

Figure 1. The polymerization process and formation of selective cavities in capillary column.

Efficiency of the Monolithic Column on the Phenolics Separation using CEC

Effect of Porogen Composition

The ability of a liquid to flow through the polymeric monoliths is essential to all their applications. This permeability is due to network of the large canal like pores which traverse the length of the macroporous monolith [21]. The composition of the porogen mixture of the pre-polymerization mixture allows the complete control of these porous properties and flow velocity.

Because of monolithic columns being packed columns rather than being filled columns, particle sizes are not considered. Instead of this, pore sizes in monolithic columns which can be adjustable with polymerization conditions are taken into account and it is considered to affect chromatographic efficiency. Actually, former studies have shown that the flow passing through pores affect the separation performance.

Taking this important point into account, monolithic columns that can make the injected fluid pass and have the ability for the optimum flow was prepared in different conditions using MIPs which have the porosity. After that, the flow efficiency of CEC column was analyzed. Solvents were selected among the nonpolar solvents and their mixtures that make hydrogen bonds and π-π interactions in a high level. The rate and state of flow effectiveness of selected solvents are given in Table 1. As can be seen from the table, when solvent mixtures prepared by adding isooctane (D and E) were used, flow was observed inside the column. The flow optimization is related to high porosity and this was obtained by using isooctane at 5% level (D). Also, when isooctane volume is higher than 10%, the solubility of monomer problem was occured. However, separation of seven different phenolic species was not achieved at E, complete separation was observed at D. For this reason, the mixture analyzed D was approved and used in the experiments.

Table 1. The effect of solvent mixtures in different ratios on the flow.

	Acetonitrile	Toluene	Isooctane	Flow
A	70	30	-	-
B	30	70	-	-
C	20	80	-	-
D	10	85	5	Flow
E	10	90	10	Flow

These columns assembled into CEC system were used for the separation of seven different phenolic compounds. As can be seen from Figure 2, monolithic column that has been prepared using 80/20% asetonitrile/phosphate buffer with only 85/5% toluene/isooctane porogen mixture at 15 kV voltage can make separation effectively. Despite the fact that flow is good in 80/10 % toluene/isooctane mixture, the separation is not good.

Effect of the Mobile Phase

Flow speed and separation is related to the mobile phase combination. Since, the allotropic force sequence is MeOH < THF < Urea < Acetonitrile < Dioxane [22], acetonitrile has chosen as an organic solvent. It has appointed that pH 7.0 has the best separation property among the separations done at the prepared phosphate buffers at the pH 5.0-8.0 range. Eluent compositions were prepared at this pH to provide best separation and as can be seen from the Figure 3, seven different phenolic compounds were completely separated at the acetonitrile/phosphate buffer ratios as 80/20.

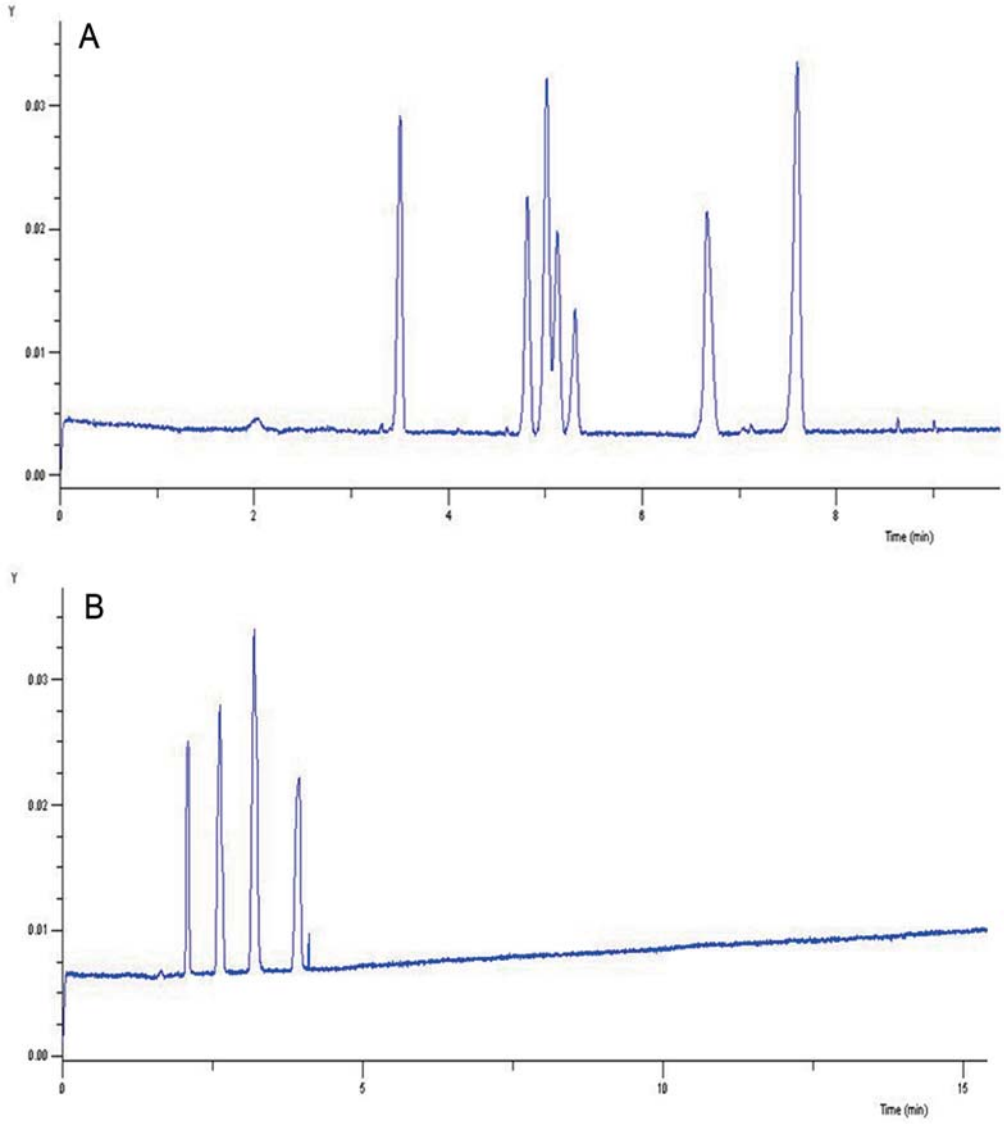

Figure 2. The effect of toluene/isooctane mixture prepared at different ratios on the separation: toluene/isooctane % A. 85/5, B. 80/10.

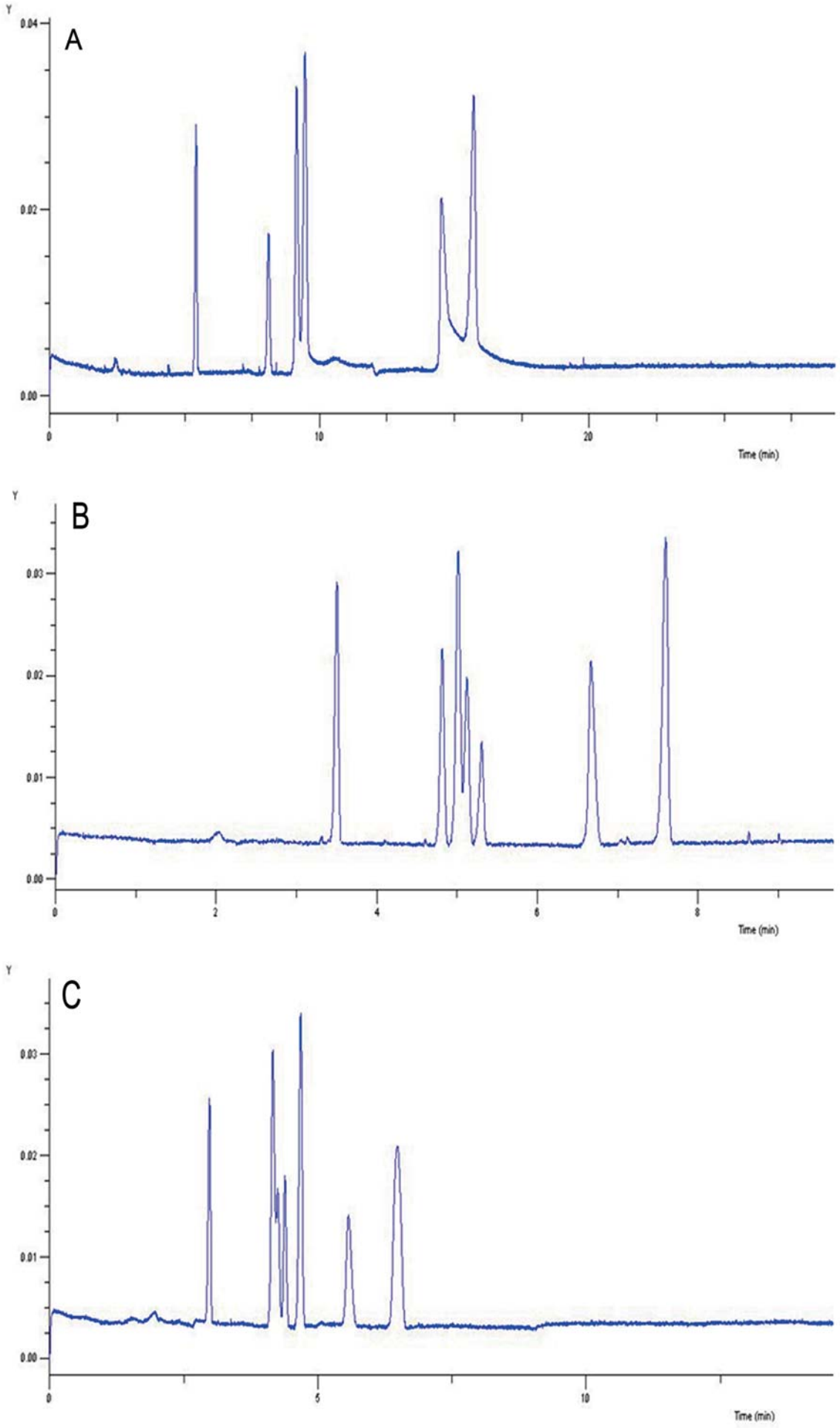

Figure 3. The effect of acetonitrile/phosphate buffer mixture prepared at different ratios on the separation: acetonitrile/phosphate % A. 70/30, B. 80/20, C. 90/10.

Effect of Applied Voltage

Performed experimental studies showed that amount of voltage applied to the CEC system is effective on the separation. For this purpose, the applied voltage was varied from 10 to 30 kV. The optimal voltage was selected as 15 kV by considering effective separations of phenolic compounds (Figure 4).

Separation Efficiency of CEC Monolithic Column

Theoretical Plate Number, Resolution and Selectivity Coefficient

For the investigation of whether the monolithic column that has prepared using 85/5 % (Toluene/Isooctane) under UV light can be used for the determination of phenolic compounds, some theoretical calculations were applied. Not only linear flow speed's being effective in the smaller pores, but also theoretical plate number (N) is an effective element. N is related to half width of peak obtained with retention time of components measured from chromatograms;

$$N = 5.54 \, (t_R / w_{0.5})^2$$

Theoretical plate number has found to be 31220 N/M for p-nitrophenol separation. The resolution, R, of a column provides a quantitative measure of its ability to separate two analytes and defined as

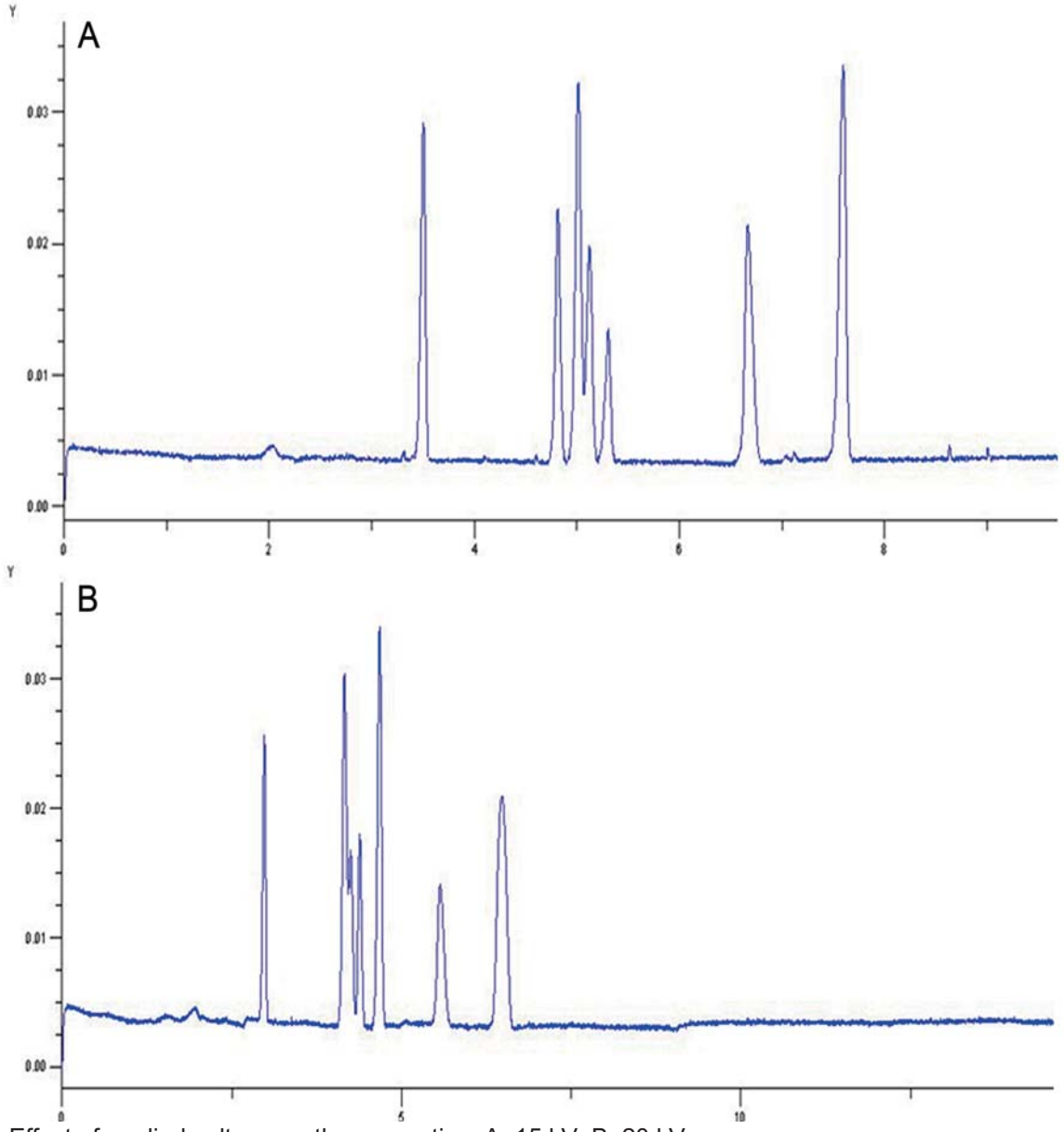

Figure 4. Effect of applied voltage on the separation; A. 15 kV B. 20 kV

$R = 2 [t_{R,A} – t_{R,B} / t_{W,A} + t_{W,B}]$

$t_{W,A}$ and $t_{W,B}$ are the widths of the two adjacent peaks at the baseline. At a resolution of 1.0, A contains about 2 % B and B contains about 2 % A. At a resolution of 1.5, the overlap is 1 % and a resolution of 1.5 gives a complete separation of A and B where as a resolution of 0.75 does not. In Table 2, the R values of p-nitrophenol, p-chlorophenol, o-nitrophenol, dichlorophenol, o-cresophenol and phenol have been given compared to each other. As can be seen from the table, R values except for dicholorophenol/o-cresophenol is higher than 1.0, even 1.5. Also, this shows that the separation and determination of phenolic compounds are possible.

Table 2. The Resolution Values of p-nitrophenol, p-chlorophenol, o-nitrophenol, dichlorophenol, o-cresophenol and phenol with respect to each other.

	R
p-nitrophenol / p-chlorophenol	4.278
p-chlorophenol / o-nitrophenol	6.857
p-nitrophenol / dichlorophenol	1.193
dichlorophenol / o-cresol	0.957
o-cresol / m-cresol	1.638
m-cresol / phenol	9.163

The degree of separation of two peaks is related to the separation factors shown as α. Selectivity factor is also known as relative retention and described with

$A = t_{R,B} – t_0 / t_{R,A} - t_0] = k_B / k_A$

t_0 is the retention time of acetone selected as void marker for monolithic column and its value is 0.617. In this equivalence, k_b is the capacity factor of species bound strongly on the column also k_a is the capacity factor of the slower-moving or weakly hold species on the column. The more closer α value to the 1.0, the more difficult become two peaks separation from each other. Using the equivalence above, the capacity factor of p-nitrophenol with respect to the p-chlorophenol, o-nitrophenol, dichlorophenol, o-cresophenol and phenol was calculated (Table 3).

Table 3. Separation Factor Values of p-nitrophenol according to the p-chlorophenol, o-nitrophenol, dichloropenol, o-cresol, m-cresol and phenol.

	A
Kp-nitrophenol / Kp-chlorophenol	1.167
Kp-nitrophenol / Ko-nitrophenol	1.497
Kp-nitrophenol / Kd-chlorophenol	1.553
Kp-nitrophenol / Ko-cresol	1.593
Kp-nitrofenol / Km-cresol	1.675
Kp-nitrofenol / Kphenol	2.396

Looking through the separation factors, it can be said that π-π interaction and created cavity for the target is very effective. Because of these properties, the phenolic compounds show the following selectivity order: p-nitrophenol > p-chlorophenol > o-nitrophenol > dichlorophenol > o-cresol > m-cresol > phenol. The chloro and nitro groups which are found in the structure of p-nitrophenol, p-chlorophenol and dichlorophenol attract the electrons on benzene ring remaining the benzene ring as electron deficient. For all that, phenyl groups which have located in the functional monomer are interacted with these electron deficient sites and in this way the selective cavities are formed. Also, this factor makes the separation more effective.

CONCLUSION

It should be noted that the contamination of drinking water by phenolic compounds at even a

concentration of 1 mgL^{-1} could bring about significant taste and odor problems making it unfit for use. Seven phenols which were selected in the present study: phenol, methyl phenols (o- and m-cresol), chloro phenols (p-dichloro phenol and 2,6-dichlorophenol) and nitrophenols (p- and o-nitrophenol) belong to a group of alkyl substituted phenols commonly found in fuel and petrochemical products with similar chemical properties.

As mentioned in a detailed way and results were given above, phenol imprinted polymer which has prepared in this study is a very selective material for the determination of toxic phenol compounds especially. The characterization of monomer, polymer and column which have prepared using this material shows that 4-nitrophenol imprinting has been performed successfully. Also, optimum conditions considering the effects of porosity, solvent, eluent, applied voltage and polymerization have been determined for MIP based CEC monolithic column and by doing this column material giving best performance has been prepared. Also, selectivity coefficients of p-nitrophenol, p-chlorophenol, o-nitrophenol, dichloropenol, o-cresol, m-cresol ve phenol compounds using 4-nitrophenol imprinted monolithic column were calculated. Obtained results with the existence of compounds like p-nitrophenol, p-chlorophenol, o-nitrophenol, dichloropenol, o-cresol, m-cresol ve phenol showed that the material especially selective to imprinted p-nitrophenol. For this reason, this study has important results not only in the area of developing chromatographic materials and sensor but also removal of toxic species and determination of their amount.

ACKNOWLEDGMENTS

This work has been supported by Turkish Science and Technology Research Council (TUBITAK-MISAG-235). Furthermore, it has dedicated to the 50th anniversary of Anadolu University.

REFERENCES

1. S. Rengaraj, S-H. Moon, R. Sivabalan, B. Arabindoo and V. Murugesan, J. Hazardous Materials, B89 (2002) 185.
2. R.M. Alanso-Salces, E. Korta, A. Barranco, L.A. Berrueta, B. Gallo and F. Vicente, J. Chromatography A, 933 (2001) 37.
3. G. Wulff, Angew. Chem. Int. Ed. Engl., 34 (1995) 1812.
4. A.A. Özcan, R. Say, A. Denizli and A. Ersöz, Analytical Chemistry, 78 (2006) 7653.
5. E. Birlik, A. Ersöz, A. Denizli and R. Say, Anal. Chim. Acta, 565 (2006) 145.
6. S. Büyüktiryaki, R. Say, A. Denizli and A. Ersöz, Talanta, 71 (2007) 699.
7. M.A. Rodriguez-Delgado, S. Malovana, J.P. Perez, T. Borges and F.J.G. Montelongo, J. Chromatography A, 912 (2001) 249.
8. A. Escarpa and M.C. Gonzales J. Chromatogrphy A, 897 (2000) 161.
9. M. Rizz , D. Ventrice , M.A. Varone , R. Sidari and A. Caridi, Journal of Pharmaceutical and Biomedical Analysis, 42 (2006) 46.
10. M. Rubilar, M. Pinelo, C. Shene, J. Sineiro and M. Nunez, J. Agric. Food Chem., 55 (2007) 10101.
11. I. Rodriguez, M.I. Turnes, M.H. Bollain, M.C. Mejuta and R. Cela, J. Chromatography A, 778 (1997) 279.
12. A. Dermaux and P. Sandra, Electrophoresis, 20 (1999) 3027.
13. K.D. Bartle and P. Myers, J. Chromatography A, 916 (2001) 3.
14. D. Lima, A.C. Duarte and V.I. Esteves,Talanta, 72 (2007) 1404.

15. F.P. Capote, J. Rodriguez and M. Castro, Journal of Chromatography A, 1139 (2007) 301.

16. F.N. Fonseca, M. Tavares and C. Horvath, Journal of Chromatography A, 1154 (2007) 390.

17. Z-S. Liu, Y-L. Xu, C. Yan and R-Y. Gao, J. Chromatog. A., 1087 (2005) 20.

18. I. Steinke, D.C. Sherrington and I. R. Dunkin, Adv. Polym. Sci., 123 (1995) 81.

19. B. Sellergren, Techniques and Instrumentation in Analytical Chemistry, Molecularly Imprinted Polymers, 1st edn., Vol 23, Chapter 6, Elsevier, 2001.

20. A. Ersöz, A. Denizli, İ. Şener, A. Atılır, S. Diltemiz and R. Say, Sep. Purif. Technol., 38 (2004) 173.

21. E.C. Peters, M. Petro, F. Svec and J.M.C. Frechet, Anal. Chem., 69 (1997) 3646.

22. Z. Liu, H. Zou, M. Ye, J. Ni and Y. Zhang, Electrophoresis, 20 (1999) 2898.