

# Comparative Study of the Retention of Nine Phenolic Compounds on Anionic Exchanger Resins

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Received 18 February 2004

The retention efficiency of phenol and eight derivative compounds on both Sephadex and Dowex anionic exchangers is evaluated, including chlorophenols (2-chlorophenol, 4-chlorophenol, 4-cresol), nitrophenols (2-nitrophenol, 4-nitrophenol, 4-methyl-2-nitrophenol), and naphthols (1-naphthol and 2-naphthol). The retention efficiency of these phenolic compounds on Sephadex QAE A-25, Sephadex DEAE A-25, and Dowex 1 × 8 (200–400 “mesh”) is studied from solutions containing all these compounds simultaneously. Mixtures of the compounds are passed in-line through mini-columns of these polymeric supports by means of a peristaltic pump and the variables influencing the retention of phenols on the supports are studied for each compound as well as the nature, concentration, and volume of the eluent. The separation, identification, and determination of the eluate composition was carried out by HPLC and the detection of the analytes was performed using a dual UV detector tuned at 215 nm and 230 nm. Results showed different levels of retention efficiency.

Phenol and substituted phenols such as mono- and disubstituted chloromethyl and nitro derivatives and those containing two aromatic condensed rings belong to the more toxic and common contaminants from a wide variety of industries sources (plastics, industrial dyes, and particularly from pulp processing [1]). Biodegradation products of humic acids, tannins and lignins also produce phenolic compounds [2]. Chloro- and nitrophenols are also degradation products of chlorinated phenoxy acid herbicides and organophosphorus pesticides [3].

Concentration of a few  $\mu\text{g dm}^{-3}$  can affect the taste and odour of water and fish. For this reason, a number of phenolic compounds are listed in the European Community Directive and the US Environment Protection Agency (EPA) [4–7] concerning dangerous compounds. Levels of phenolic compounds in surface waters for drinking purpose should be below  $10 \mu\text{g dm}^{-3}$  according to E.C. Directive 75/440/EEC [8]. So, the determination of these compounds at  $\mu\text{g dm}^{-3}$  level is of great interest. Usually, chromatographic methods are used [9, 10] after a step of preconcentration. Solid Phase Extraction (SPE) is the procedure most used to preconcentrate phenolic compounds and it has replaced sorbent extraction so avoiding the use of toxic sorbents [11] and improving the reproducibility of the measurements [12].

Several sorbents have been used in the reten-

tion and preconcentration of phenolic compounds. Bonded silica is one of the most popular for a wide range of polarity and it has been applied to retention and preconcentration of phenol and chlorophenols [13–15]. Styrenedivinylbenzene copolymers have high efficiency towards nonpolar and medium-polarity molecules and some phenolics have been preconcentrated such as nitrophenols [16–19].

Silicagel-C<sub>18</sub> has also been used to determine the eleven priority phenolics [15, 20–22]. The use of cyclohexyl sorbent and ion-pair reagents has also been demonstrated to have good recoveries for phenol [13, 22]. Anion-exchange resins can be used for this purpose due to their capability to retain them under their anionic form by ion exchange and also by adsorption, mainly in polymeric resins with an aromatic matrix.

Nevertheless, to the best of our knowledge, no comparative studies have been described about the behaviour of anion-exchange resins towards phenolics. Thus, in this paper, a comparative study of the efficiency of different anion-exchange sorbents (*i.e.* Sephadex QAE A-25, DEAE A-25, and Dowex 1 × 8) for nine phenolics solid-phase retention has been carried out. The study is focused to know the capability of these sorbents to retain the nine compounds and the effect of variables influencing the retention is described. An HPLC method was used to follow this study.

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## EXPERIMENTAL

All reagents were anal. grade and deionized water was used to prepare all solutions.

The phenolic compounds were supplied by Sigma (Alcobendas, Madrid) (2-chlorophenol (2-CP), 4-chlorophenol (4-CP), 4-cresol (4-MP), 2-nitrophenol (2-NP), 4-nitrophenol (4-NP), and 1-naphthol (1-NPH)), Panreac (Barcelona) (phenol (PH) and 2-naphthol (2-NPH)), and Aldrich (Madrid) (4-methyl-2-nitrophenol (4-M-2-NP)).

Aqueous stock solutions containing  $200 \text{ mg dm}^{-3}$  of the nine phenolic compounds simultaneously were prepared and stored at  $4^\circ\text{C}$ . Working solutions ( $\rho = 0.20\text{--}5.0 \text{ mg dm}^{-3}$ ) were prepared daily by suitable dilution with deionized water.

For the mobile phases, acetonitrile (Fluka, Madrid) and phosphoric acid (Panreac, Madrid) were used.

Sephadex QAE A-25 and DEAE A-25 ion-exchange gel (Aldrich, Alcobendas, Madrid) were used as supplied. Dowex ( $1 \times 8$ ) 200—400 resin (Aldrich, Alcobendas, Madrid) was washed several times with deionized water, then treated with 2 M-HCl for 4 h and finally with deionized water until the wash was free from chloride. Then it was air-dried and stored in a polyethylene container.

Liquid chromatograph Model 600 E Waters (Mildford, Massachusetts, U.S.A.) with Column Superspher 100 RP-18  $\text{C}_{18}$  stainless steel,  $250 \text{ mm} \times 4 \text{ mm}$ ,  $5 \mu\text{m}$  (Merck, Darmstadt, Germany) was used. Absorbance detector UV VIS Dual  $\lambda$  Waters 2487 (Mildford, Massachusetts, U.S.A.) was used to perform spectra of eluate and absorbance measurements at 215 nm and 230 nm.

Gilson Minipuls-3 peristaltic pump (Villiers Le Bel, France) and pH-meter Crison 2000 digital Selecta (Barcelona, Spain) were used. Absorbance measurements to determine the break-through volume were performed at 230 nm with a Lambda 2-UV VIS spectrophotometer (Perkin—Elmer, Bucks, UK) which was interfaced to a compatible PC running Perkin—Elmer Computerized Spectroscopy Software (PECCS V 4.1).

## Retention and Elution System Procedure

$30 \times 3 \text{ mm}$  i.d. glass minicolumns were prepared by packing the respective sorbent with the aid of the peristaltic pump. The minicolumns were conditioned by passing through them  $5 \text{ cm}^3$  of aqueous solution of HCl and NaOH at the different pH values assayed (2—12). Then,  $3 \text{ cm}^3$  of the phenolic mixture were pumped through the columns, packed with Sephadex QAE A-25 and DEAE A-25 ion-exchange gel and Dowex ( $1 \times 8$ ) 200—400 resin. Next,  $5 \text{ cm}^3$  of eluents of different nature (depending on the sorbent) were pumped in order to elute the analytes. The eluates were kept at  $4^\circ\text{C}$  until separation and determination of their composition performed by using HPLC.

## Chromatographic Conditions

HPLC chromatographic separation was carried out using a mobile phase: solvent A, acetonitrile and solvent B, phosphoric acid. The flow rate was  $1 \text{ cm}^3 \text{ min}^{-1}$  and the sample volume injected was  $20 \text{ mm}^3$ . The working column temperature was  $20^\circ\text{C}$ . The de-

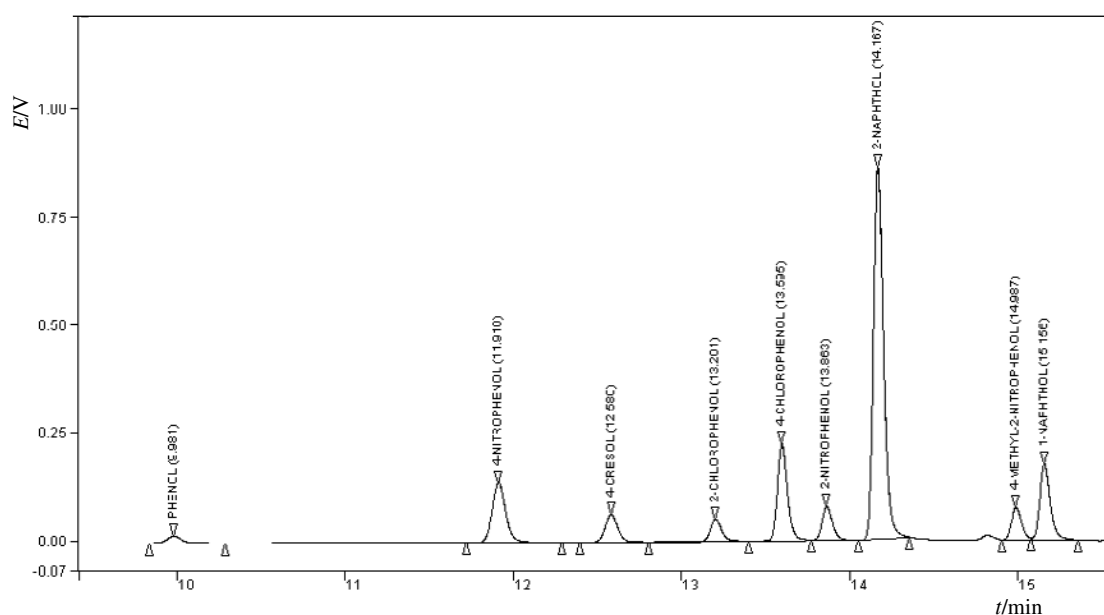


Fig. 1. Liquid chromatogram obtained by the developed method showing the separation of the nine phenolic compounds. Conditions: detector 230 nm;  $\rho(2\text{-NPH})$ :  $1.12 \text{ mg dm}^{-3}$ ,  $\rho(\text{rest of compounds})$ :  $3 \text{ mg dm}^{-3}$ .

**Table 1.** Analytical Parameters of the HPLC Method

Phenolic compound	Correlation coefficient $r$ ( $n = 5$ )	Intercept	Slope	RSD/%	DL/(mg dm <sup>-3</sup> )	
					Test I	Test II
Phenol	0.9995	2236	25129	1.80	0.17	0.05
<i>p</i> -Nitrophenol	0.9992	1560	18769	1.71	0.22	0.06
<i>p</i> -Cresol	0.9991	952	19581	1.67	0.24	0.07
2-Chlorophenol	0.9992	450	19294	1.90	0.22	0.06
4-Chlorophenol	0.9999	4017	25182	1.78	0.07	0.02
<i>o</i> -Nitrophenol	0.9999	3632	27023	2.35	0.08	0.02
2-Naphthol*	0.9968	364	156552	3.24	0.10	0.03
4-Methyl-2-nitrophenol	0.9999	1130	40413	4.47	0.09	0.02
1-Naphthol	0.9964	15501	47941	3.50	0.46	0.13

Concentration ranges: (1.0—5.0) mg dm<sup>-3</sup>; \*(0.23—1.12) mg dm<sup>-3</sup>. Test I from [24]; Test II from [25];  $DL = \frac{S_{y/x}}{b} \sqrt{\frac{n-2}{n-1}}$ .

tection wavelength was set to 215 nm for PH, 2-CP, 4-MP, 1-NPH, 4-M-2-NP, and 2-NP and 230 nm for 4-CP, 2-NPH, and 4-NP.

The gradient profile at initial conditions was 20 % A and 80 % B during 7 min; then changed linearly to 45 % A and 55 % B; at 9.5 min was changed linearly to 80 % A and 20 % B. After the chromatography was performed (15.5 min), the mobile phase was returned to initial conditions. This method was developed and adapted from a Varian Application HPLC procedure [23]. Fig. 1 shows a liquid chromatogram obtained by the developed method and the separation of the nine phenolic compounds for a detection wavelength of 230 nm.

## RESULTS AND DISCUSSION

Standard calibration graphs for the compounds of the system were constructed by plotting the peak area produced by injection of triplicate of solutions in the following concentration ranges: 0.23—1.12 mg cm<sup>-3</sup> for 2-NPH and 1.0—5.0 mg cm<sup>-3</sup> for all the other compounds. Calibration curves were obtained by regression analysis and the correlation coefficient ( $r$ ), slope, and intercept for each run were calculated. The detection limits (DL) for each compound were calculated according to the *Miller and Miller* [24] and *Cuadros* [25] criteria, respectively. Concentration ranges and RSD/% at two analyte concentration levels are shown in Table 1.

Phenols are weak acids according to their  $pK_a$  values [26]. Therefore, the sample pH value influences their dissociation equilibrium. So for pH values above the respective  $pK_a$  value, phenolics are expected to be under their anionic form and they can be sorbed on anion-exchange resins by an exchange mechanism.

The effect of this variable was tested from 2.0 to 12.0. Fig. 2 shows the fraction retention on all the three ion exchangers for all components as a function of the sample pH.

In general, both Sephadex resins QAE A-25 and

DEAE A-25 show higher retention percents compared with Dowex.

On one hand, the amounts retained are different for each compound depending on the sample pH. On the other hand, at a given pH the retention varies from a compound to another, the resin Sephadex QAE A-25 retaining a higher number of analytes for all the pH values assayed and Dowex showing the lowest number of phenolics retained. Anionic resin Dowex seems not to work as Sephadex resins probably due to its cross-linkage it does not contribute to the homogeneous diffusion of the compounds through it.

The percent average values of retained phenolics are higher for DEAE A-25 than for QAE A-25 for all pH values tested, except for pH 2.0.

At acid pH, all resins retain the lowest average amount of phenolics, QAE A-25 showing the highest percent average of all the three resins (20.0 % *vs.* 4.5 % and 5.1 % for DEAE A-25 and Dowex 1 × 8, respectively).

At basic pH values a substantial increase is observed, in general, in the average retained amounts in both Sephadex resins, DEAE A-25 showing a higher increase (36.0—42.0 %) than QAE A-25 (28.0—35.0 %).

On the other hand, Dowex resin shows low retention percents (6.5 %) at slightly basic pH values. At high pH values, the average of retained amounts increases reaching a similar value to that obtained for Sephadex resins (33 %) but a lower number of phenolics are retained. These results seem to indicate that the retention on this support at these values of pH is due to an ion-exchange process, as the phenolics are completely dissociated in these operating conditions.

The influence of flow rate on the retention was studied by varying it from 0.6 to 1.6 cm<sup>3</sup> min<sup>-1</sup>. Flow rates above 1.6 cm<sup>3</sup> min<sup>-1</sup> caused overpressure problems in the system. 3 cm<sup>3</sup> of a sample solution containing 1 mg cm<sup>-3</sup> of each analyte (except PH, which had a concentration of 3 mg cm<sup>-3</sup>) was passed through the column. Then, 3 cm<sup>3</sup> of a 0.15 M-KCl solution was

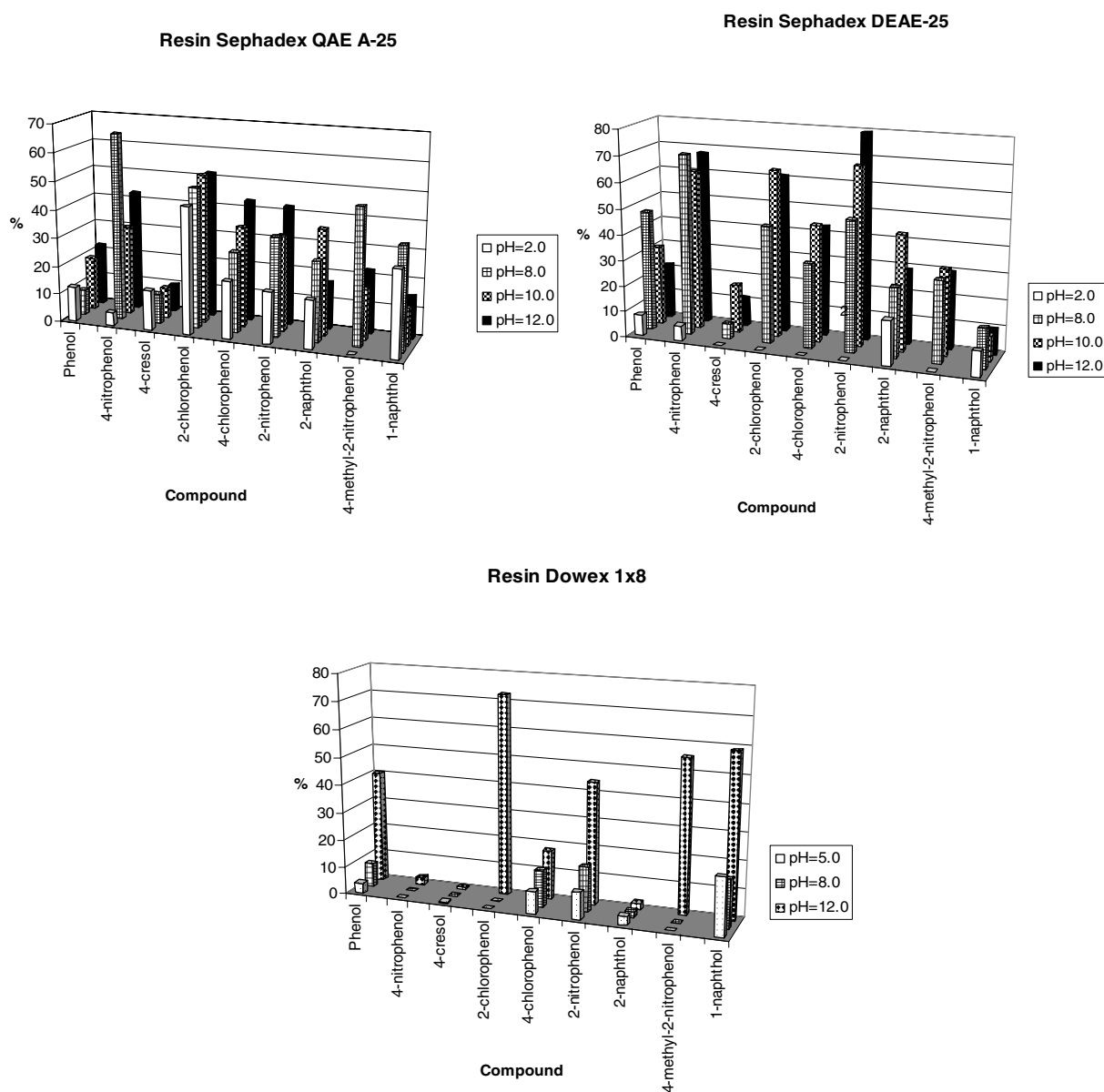


Fig. 2. Influence of pH on retention percent of phenolic compounds.

used to elute all phenolic compounds. A flow rate of  $1 \text{ cm}^3 \text{ min}^{-1}$  was selected, as higher flow rates values caused a decrease in the fraction retention of each analyte, due to a lower contact time between the analyte and the ion exchanger.

The break-through volume was determined by passing a solution mixture ( $\text{pH} = 12.0$ ) of the nine phenolic compounds containing  $1 \text{ mg dm}^{-3}$  of each analyte ( $3 \text{ mg dm}^{-3}$  for PH) at a flow rate of  $1 \text{ cm}^3 \text{ min}^{-1}$ . After passing through the column, a spectrophotometer furnished with a quartz flow cell of  $1 \text{ cm}$  optical path length was used for continuous monitoring of the absorbance signal at  $230 \text{ nm}$ . In all cases, the break-through volume was  $4 \text{ cm}^3$  of the above indicated solution, as from this volume the absorbance signal kept constant.

Optimization of elution was performed by passing  $3 \text{ cm}^3$  of the working phenolic compounds solution ( $\text{pH} = 12.0$ ) through the conditioned minicolumns. The nature of the eluting solutions assayed was based on the support characteristics. For Sephadex resins, NaOH and KCl solutions were tested in the concentration ranges  $0.05\text{--}0.20 \text{ mol dm}^{-3}$ . NaOH solutions caused compaction and alteration of the resins, so they were discarded.  $0.10 \text{ M-KCl}$  solution was selected, as it provided the complete elution of all phenolic compounds retained by using  $3 \text{ cm}^3$  of it.

For Dowex  $1 \times 8$  resin,  $0.15\text{--}1 \text{ M-KCl}$  solution and methanol-KCl mixtures ( $\varphi_{\text{r}} = 50 : 50$  and  $25 : 75$ , respectively) were tested. The complete elution for all compounds was achieved using  $1 \text{ M-KCl}$  solution except for 2-NP and 4-MP. The complete elution of

these two compounds was obtained by using 2 cm<sup>3</sup> of methanol—KCl solutions,  $\varphi_r = 25 : 75$  and  $50 : 50$ , respectively.

## CONCLUSION

The experimental variables conditioning the behaviour of three anion-exchange polymeric sorbents towards the retention of the phenolic mixture were studied. A Varian (Application 1209 HPLC) method with UV dual detection was adapted to perform this study in order to separate and quantify phenolics in eluates. Satisfactory resolution of the mixture was obtained. The sample pH value did not have any influence in the HPLC separation.

The retention efficiency was not the highest at the same time for neither of the pH values. Nevertheless, from the results obtained the use of resin Sephadex DEAE A-25 can be recommended for retaining, in general of a higher number of compounds with a higher efficiency adjusting the sample pH to 12.0. From this study, the pH value proposed for the retention is 12.0. Concerning the elution of the compounds of the mixture a KCl solution was necessary at different concentrations depending on the resins. The recommended flow rate for the concentration levels of this study was, in general, 1 cm<sup>3</sup> min<sup>-1</sup> for all of the three solid supports. The optimum volume eluent was 3 cm<sup>3</sup> for Sephadex and 2 cm<sup>3</sup> for Dowex resins, respectively.

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