THE XXIX SYMPOSIUM
‘CHROMATOGRAPHIC METHODS OF INVESTIGATING THE ORGANIC COMPOUNDS’

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INDIRECT DETECTION IN ION EXCLUSION CHROMATOGRAPHY

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Ion Exclusion Chromatography

Ion-exclusion chromatography (IEC) finds application in the analysis of weak and medium strength acids [1]. The detection techniques include direct UV absorbance, refractive index (RI), potentiometric, conductivity as well as mass spectrometry. The direct UV absorption at 210 nm is the most frequently used detection method for volatile fatty acids. Its detection limit is rather high because of lacking a chromophore by solute. From the other side, due to its sensitivity to even trace amounts of contaminants, it cannot be used for complex samples without suitable sample pretreatment because of interference problems. The most commonly used in IEC conductometric detector also is characterized by high sensitivity because of small dissociations of acids, additionally depressed by the buffer.

Indirect Detection

Indirect photometric detection has been already successfully applied in ion chromatography as well as and capillary electrophoresis [2]. In this case mobile phase contains an absorbing reagent of the same electric charge as analyzed solute, characterized by high molar absorption coefficient.

Objective

In the presentation it was confirmed the possibility of application of indirect detection in ion exclusion chromatography. In this case aromatic acids can be used as eluents. Two detection systems are discussed, photometric and conductometric. Derived equations as well as experimental data show indirect conductometric detection for solutes which ionic conductivities (diffusion coefficients) are smaller then background electrolyte. Direct detection (positive peaks) is observed in the opposite case. It was possible to obtain direct and indirect detection on one chromatogram, depending on the relative limit conductivity (diffusion coefficient) of solute and background electrolyte. Quantitative correlation between derived equation and experimental results was found. Phthalic acid, used as indirect detection
probe, decreased retention of aliphatic fatty acids because of the competition on adsorption sites.

**Materials and Methods**

Measurements were performed on a chromatograph consisting of Dionex (USA) GP40 gradient pump, AD20 Absorbance detector, ED40 electrochemical Detector, LC30 chromatography oven, PeakNet 5.1 chromatographic data acquisition and analysis, Tosoh Haas (Japan) TSKGel SCX(H+) 300 x 7.8 mm I.D. column. Manual injections were performed using a 100 µl syringe (Scientific Glass Engineering, Ringwood, Australia).

**Changes of the background electrolyte concentration**

Indirect detection is based on the measurements of changes of the concentration of background (probe) electrolyte. Its electric charge should be the same as a solute. It means that in the case of the acid analysis also background electrolyte should be acid. Usually it is used diluted solution of strong, completely dissociated acid. However, optimal conditions from the detection point of view are sometimes not optimal for separation. Changes of the background electrolyte concentration can be calculated from the definition of dissociation constant and mass conservation equation. For diluted solutes we can assume that the concentration of hydrogen ions is constant along the column: \([H^+] = \text{const}\). In this case, outside solute region (chromatographic peak) electroneutrality condition can be expressed as:

\[
[H^+] = \text{[B-]}. \tag{1}
\]

Tested acidic solute, HR, injected onto the chromatographic column decrease concentration of the dissociated form of background electrolyte. According to electroneutrality condition in the peak maximum, in the mobile phase:

\[
[H^+] = \text{[B-]max + [R-]}, \tag{2}
\]

where: B- and R- denote dissociated forms of the background electrolyte and solute, respectively.

Equations (1) and (2) can be easily transformed to:

\[
\text{[R-]} = \text{[B-]} - \text{[B-]max}. \tag{3}
\]

**Conductometric detection**

Conductivity of the diluted electrolyte, G, is a function of its limiting ionic conductivities, \(\lambda_i\), stochiometric coefficients, \(v_i\), valence, \(z_i\), surface area of the electrodes, \(A\), and distance between them, \(l\):
For mono-monovalent acid above equation can rewritten as:

\[ G = (\nu + c + z + \lambda + \nu - c - z - \lambda) A/l. \]

In the peak maximum this conductivity depends also on the solute concentration:

\[ G_{\text{max}} = (\lambda B^-[B^-] + \lambda H^+[H^+] + \lambda R^-[R^-]) A/l. \]

Chromatographic peak height can be calculated as a difference of the conductivities obtained from the equation (6) and (5):

\[ \Delta G = (\lambda B^-[B^-] + \lambda R^-[R^-] - \lambda B^-[B^-]) A/l = (\lambda R^-[R^-] - \lambda B^-[B^-]) A/l. \]

From equations (3) and (7) finally we can obtain:

\[ \Delta G = (\lambda R^- - \lambda B^-) [R^-] A/l. \]

From the equation (8) we can read out that height of the chromatographic peak should be directly proportional to the concentration of dissociated form of analyzed acid. Additionally, it should be possible to obtain both, direct and indirect, conductometric detection on one chromatogram. Peak direction depends then on the relative limiting ionic conductivity of solute versus background electrolyte.

**Photometric detection**

According to Lambert-Beer law, the background absorbency, \( A_b \), of the acid, \( HB \), used as a buffer can be expressed as:

\[ A_b = l \varepsilon B^-[B^-] + l \varepsilon HB[HB], \]

where: \( l \) – denotes length of detector cell, \( \varepsilon B^- \) and \( \varepsilon HB \) – molar absorption coefficients of dissociated and undissociated forms of acid, respectively.

Absorption in the peak maximum based on equations (1) and (9) is described by:

\[ A_{\text{max}} = l \varepsilon B^-[B^-]_{\text{max}} + l \varepsilon HB[HB]_{\text{max}}. \]

Height of the chromatographic peak can be obtained from the equations (9) and (10):

\[ \Delta A = l \varepsilon B^-[B^-]_{\text{max}} + l \varepsilon HB[HB]_{\text{max}} - l \varepsilon B^-[B^-] - l \varepsilon HB[HB]. \]

After combining equation (11) with equation (3), using definition of the dissociation constant and assumption that the concentration of undissociated form of solute acid is constant along the chromatographic peak ([HB]_{\text{max}} = [HB]), we can obtain:

\[ \Delta A = - l \varepsilon B^-[R^-]. \]

**Estimation of [R-]**

The mass conservation equation of the solute acid can be given as:

\[ ciVi = ([R^-] + [HR])VP, \]

\[ G = (\nu + c + z + \lambda + \nu - c - z - \lambda) A/l. \]

\[ G_{\text{max}} = (\lambda B^-[B^-] + \lambda H^+[H^+] + \lambda R^-[R^-]) A/l. \]
where VP (VP = ciVi/cmax) denotes volume of the peak maximum.

From equation (13) and definition of dissociation constant we can obtain:

(14) \[ [R^-] = \frac{ciVi}{VP(1 + [H^+]/K_a)} = \frac{ciViK_a}{VP(K_a + [H^+])}. \]

After column equilibration the mass balance of the background electrolyte can be presented in the form:

(15) \[ cb = [B^-] + [HB]. \]

Because concentration of the solute is usually small and because buffer suppresses its dissociation, it can be assumed that concentration of hydrogen ions is constant along the column. Outside of the chromatographic peak concentration of the dissociated form of background electrolyte equals to the concentration of hydrogen ions, as it is described by equation (1).

After resolving quadratic equation obtained from the equations (14) – (16) finally we obtain:

(16) \[ R^+ = \frac{cV,K_a}{V_T(2K_s + \sqrt{K_s^2 + 4K_sC_s - K_s})}. \]

where peak volume is described by:

(17) \[ VP = VR(2\pi/N)^{1/2}. \]

Equations (8), (12) and (17) can be used to predict indirect conductometric as well as photometric detection. Chromatographic peak height should be directly proportional to the amount of the solute acid and increased asymptotically with the dissociation constant. The highest peak is obtained for completely dissociated acids (anions). On the other hand, peak height decreases with the increase buffered acid dissociation constant and its concentration.

**Experimental verification**

Aliphatic acids, including oxalic, malonic, formic and acetic, were selected as the test compounds. The IEC separation of the tested acids is presented on Fig. 1. As a mobile phase 1 mM solution of phthalic acid was used. Two detectors, conductometric (A) and UV-300 nm (B) were serially connected to the column. It turned out that in all cases indirect photometric detection was obtained, whereas indirect conductometric detection was obtained only for acetic acid. For other analyzed acids direct conductometric response was observed. Finally, it should be also mentioned that phthalic acid influences retention of tested acids by the competition with hydrophobic adsorption sites.
Conclusions

It was confirmed the possibility of application of indirect detection in ion exclusion chromatography. In this case aromatic acids can be used as eluents. Derived equations as well as experimental data show indirect conductometric detection for solutes which ionic conductivities (diffusion coefficients) are smaller then background electrolyte. Direct detection (positive peaks) is observed in the opposite case. Quantitative correlation between derived equation and experimental results was found. Phthalic acid, used as indirect detection probe, decreased retention of aliphatic fatty acids because of the competition on adsorption sites.

Fig.1. Ion-exclusion chromatograms of: (1) oxalic, (2) malonic, (3) formic and (7) acetic acids. Chromatographic conditions: mobile phase - 1 mM phthalic acid, flow rate - 0.5 ml/min; injected volume - 20 µl, conductometric (A) and UV-300 nm (B) detectors.

Literature

VACANT REVERSED PHASE
HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (vRP-HPLC)

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Elution HPLC

Traditionally, in elution HPLC, the analyzed sample is injected to the flowing mobile phase. As mobile phase, just a pure solvent (e.g., water) can be used, occasionally containing an extra amount of a buffer, inclusion compounds, ion-interaction reagents etc. This order was, recently, reversed in the so-called vacancy ion exclusion chromatography (vIEC) \cite{1}.

Ion Exclusion Chromatography

The characteristic feature of IEC is the same sign of the electric charge of the resin’s dissociated functional groups and of the analyzed ions. It follows that the negatively charged samples (e.g., the dissociated acidic compounds) are repulsed (or better said, excluded) from the resin, while the non-dissociated (i.e. neutral) ones can penetrate into the stationary phase. As a result, characteristic peaks with the leading (frontal) tailing are observed, when pure water is used as eluent \cite{2}.

Objective

It is interesting to note that – in certain way – a similar effect is observed in RP-HPLC, although caused by a different mechanism. In that case, ions cannot be partitioned with participation of the hydrophobic (non-polar) coverage of the silica gel matrix.

In our paper, the above statement will be experimentally confirmed and the retention mechanism is going to be described in a theoretical way. In RP-HPLC – with pure water used as mobile phase – the leading peaks of the acids are really observed. Moreover, it was shown that vacant peaks can also be obtained by means of this particular technique, however in this technique the vacant peaks are much less symmetrical then in vIEC method.
**RP-HPLC with water used as mobile phase**

Ions are generally not separated with aid of HPLC and they are eluted in the dead column volume [3]. In pure water used as mobile phase, the leading peaks of moderately strong acids are obtained, as it is shown on Fig. 1. This is due to the fact that the more diluted acid zones of the peaks are also more dissociated and therefore less retained on the column. Thus the peak maxima migrate slower along the column than their front parts It is interesting to note, that the leading peak was obtained for the completely dissociated nitric acid as well. Probably this is due to the specific solvation of the nitrite ions. The biggest agglomerates cannot penetrate into the sorbent and are therefore eluted earlier.

**Vacant RP-HPLC**

It has turned out that in v-RP-HPLC the asymmetrical (tailing) peaks are obtained and the retention time is proportional to the sample concentration (Fig. 2).

![Graph](image)

*Fig.1. Experimentally obtained peaks of different acids in water used as mobile phase. Chromatographic conditions: column with precolumn - Hibar RP-18 5 µm, 250x4 mm ID (Knauer), temperature 30°C, flow rate 1 ml min⁻¹, mobile phase water, volume injected 20µL, detector UV 210/254 nm. The analyzed acids: nitric – black, oxalic – orange, formic – blue, acetic – pink, salicylic – green, and benzoic – red.*
Fig. 2. Experimentally obtained peaks of different acids in water used as mobile phase in vacant RP-HPLC. Chromatographic conditions: column with precolumn - Hibar RP-18 5 µm, 250x4 mm ID (Knauer), temperature 30°C, flow rate 1 ml min⁻¹, mobile phase water, volume injected 20µL, detector UV 210/254 nm. The analyzed acids: oxalic – orange, formic – blue and acetic – pink.

Theoretical approach

The proposed model is based on the assumption, that the non-dissociated acid as well as its dissociated form can penetrate into the water occluded in the adsorbent pores. However, adsorption of the non-dissociated acid strongly prevails over the adsorption of the R⁻ ions. The proposed theoretical approach correctly modeled the dependency of the retention time on the analyte concentration in both the classical and the vacancy mode of RP-HPLC. The comparison between theoretical and experimental peak profiles will be presented on a poster.

References