Isotachophoresis as a preseparation technique for liquid chromatography
Schoots, A.C.; Everaerts, F.M.

Published in:
Journal of Chromatography. Biomedical Applications

DOI:
10.1016/S0378-4347(00)84853-7

Published: 01/01/1983

Document Version
Publisher’s PDF, also known as Version of Record (includes final page, issue and volume numbers)

Please check the document version of this publication:

• A submitted manuscript is the author's version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
• The final author version and the galley proof are versions of the publication after peer review.
• The final published version features the final layout of the paper including the volume, issue and page numbers.

Link to publication

Citation for published version (APA):

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying the publication in the public portal

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.
Isotachophoresis as a preseparation technique for liquid chromatography

A.C. SCHOOTS* and F.M. EVERAERTS

Laboratory of Instrumental Analysis, Department of Chemical Engineering, Eindhoven University of Technology, P.O. Box 513, 5600 MB Eindhoven (The Netherlands)

(First received December 20th, 1982; revised manuscript received May 6th, 1983)

High-performance liquid chromatographic (HPLC) profiles of uremic serum ultrafiltrate are rather complex [1]. For purposes of identification and characterization of the HPLC peaks, information may be obtained from chromatographic retention data, on-line and off-line HPLC—mass spectrometric analysis, off-line (Fourier) infrared analysis and to a certain extent from UV-ratio monitoring at multiple wavelengths [2]. However, it is desirable to decrease the complexity of the profiles, especially in view of the spectrometric identification techniques, where peak impurities might obscure the spectra. For this reason uremic serum ultrafiltrate was preseparated by isotachophoresis [3], the advantages of which are as follows. (1) The concentration effect of dilute samples. (2) The self-sharpening effect of zone boundaries. (3) The possibility of selecting a discrete amount of anions or cations by a proper choice of electrolyte conditions. (4) The length between leading zone and terminating zone (sample) is constant at the moment the terminator has passed the injection point. The steady-state therefore need not to be reached for sample collection. (5) Using valves for sample introduction even allows the collection of non-ionic compounds, as they remain in the valve during the isotachophoretic separation.

EXPERIMENTAL

Isotachophoresis

Separations were performed on an LKB Tachophor isotachophoretic analyzer (LKB, Bromma, Sweden) at 60 μA (stabilized current, end voltage 9 kV), in a 0.4 mm I.D. PTFE capillary instead of the original separating capillary plate. Test runs were also done in home-made equipment [3] using both UV and conductivity detection.
Amaranth red and fluorescein were used as coloured markers in the initial experiments. Hard-cutting of the zone train migrating between amaranth red and fluorescein or terminator was done by means of a razor blade [4].

In the preparation runs, 1 μl of uremic serum ultrafiltrate was injected into the isotachophoretic analyzer. The volume collected by hard-cutting (5 μl, 4 cm) was transferred to a conical microvial (Chrompack, Middelburg, The Netherlands) and injected into the liquid chromatograph using a 10-μl syringe (Glenco, Chrompack, Middelburg, The Netherlands).

So far no hard-cutting has been performed using a PTFE valve as described by Kenndler and Kanianský [5].

**Liquid chromatography**

The equipment used consisted of two Model 100 A pumps, a Model 321 controller, and a Model 160 fixed-wavelength UV detector, all from Beckman (Berkeley, CA, U.S.A.). A 1-μl aliquot of serum ultrafiltrate was diluted to 5 μl and injected into the liquid chromatograph. Further experimental conditions for isotachophoresis and liquid chromatography are given in Table I.

In this way the same absolute amounts of (anionic) solutes in the serum with and without isotachophoretic preseparation are loaded on the HPLC column. Additional experimental conditions for isotachophoresis and liquid chromatography are given in Table I.

**TABLE I**

**OPERATIONAL SYSTEM FOR ISOTACHOPHORETIC AND LIQUID CHROMATOGRAPHIC ANALYSES**

<table>
<thead>
<tr>
<th>Isotachophoresis</th>
<th>Electrolyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leading</td>
<td>Terminating</td>
</tr>
<tr>
<td>Anion</td>
<td>Concentration</td>
</tr>
<tr>
<td>Chloride</td>
<td>0.025 M</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.025 M</td>
</tr>
</tbody>
</table>

**Liquid chromatography**

<table>
<thead>
<tr>
<th>Mobile phase</th>
<th>100% solvent I to 100% solvent II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gradient</td>
<td>Within 30 min</td>
</tr>
<tr>
<td>Solvent I</td>
<td>0.05 M ammonium formate pH 4—methanol (95:5, v/v)</td>
</tr>
<tr>
<td>Solvent II</td>
<td>methanol</td>
</tr>
<tr>
<td>Column</td>
<td>25 cm x 4.6 mm, stainless steel, packed with Polygosil-60, C18, 5-μm particles**</td>
</tr>
<tr>
<td>Detection</td>
<td>UV at 254 nm</td>
</tr>
<tr>
<td>Flow-rate</td>
<td>1 ml/min</td>
</tr>
</tbody>
</table>

*HEPES = N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid, sodium salt (Sigma, St. Louis, MO, U.S.A.).
Uremic serum ultrafiltrate

Uremic serum ultrafiltrate was obtained from Prof. S. Ringoir, Department of Nephrology, University Hospital of Ghent, Belgium. The filtrate became available during a sequential ultrafiltration–hemodialysis artificial kidney treatment of a uremic patient. Large molecules such as proteins are rejected by the artificial kidney membrane (molecular weight cut-off 10,000) and consequently are not present in the serum ultrafiltrate.

RESULTS

Fig. 1 shows an isotachophoretic test run of a uremic serum ultrafiltrate sample. Test runs were performed on home-made equipment using both UV and conductivity detection. From these runs zone train lengths between amaranth red as frontal coloured marker and fluorescein, useful as a terminal marker, or terminator were simply determined. Therefore, in the isotachopherogram of Fig. 1 only amaranth red is present.

Fig. 1. Anionic separation of uremic serum ultrafiltrate (0.5 µl injected) by isotachophoresis (test run). UV absorption and conductivity detector traces are shown (R = resistance). a = Amaranth red; b = phosphate; c = hippurate; d = urate. Conditions are given in Table I.

Hard-cutting was done on the 0.4 mm I.D. capillary in the LKB apparatus, between amaranth red and the terminator, of which the position of the zone boundary was calculated from the test runs performed. Fluorescein was not used here to prevent interference in the HPLC analysis.

In Fig. 2 the HPLC profiles of uremic ultrafiltrate with and without anionic preseparation are compared. After anionic preseparation a number of peaks in the HPLC profiles have disappeared. These are either cationic or neutral constituents.

From the chromatographic retention data tentative peak assignments have been made for some major peaks, as given in the figure legend.
DISCUSSION

From the experiments it can be concluded that the combination of isotachophoresis and liquid chromatography can give valuable information about the identity or character of solutes in a complex diverse matrix such as biological fluids.

In this study proteins were not present in the samples, but they can be readily separated from the anionic or cationic low molecular weight solutes in the sample in the same isotachophoretic (pre)separation run [6], by choosing suitable operational conditions.

Combination of the selectivities of isotachophoresis and HPLC makes a powerful combination. In isotachophoresis a choice is made between anionic and cationic preseparation. Variation of the pH of the leading electrolyte influences the mobility of the different species, as they have different pK values. In HPLC selectivity can be influenced by the nature of both the mobile phase and the stationary phase in a most flexible way.

Direct transfer of the aqueous samples from isotachophoresis to HPLC imposes the use of reversed-phase liquid chromatography. However, isotachophoresis in non-aqueous media, which is at present being developed [7], will be compatible with normal-phase liquid chromatography as well. With some technical developments that are available or will be available in the near future [5, 8] it might be possible to select more discrete regions of the migrating zone train. These regions in capillary isotachophoresis necessarily represent small sample volumes (< 1 μl). Combination with microbore liquid-chromatography columns (e.g. 1 mm I.D.) therefore seems promising. As the chromatographic
dilution in the microbore columns (1 mm I.D.) is much less than in wide-bore columns (4.6 mm I.D.), the former will have a higher mass sensitivity by a factor of 20. This will be an advantage in those cases where only small sample volumes are available, because in wide-bore columns larger sample volumes can be injected.

The on-line coupling of the techniques of isotachophoresis and microbore liquid chromatography and microbore liquid chromatography and mass spectrometry are at present under investigation.

REFERENCES