Journal of Chromatography A, 1305 (2013) 188–192

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Mechanism of formation of system peaks in ion-exclusion chromatography

Milko Novič[a,*], Paul R. Haddad[b]

[a] Faculty for Chemistry and Chemical Technology, University of Ljubljana, Aškerčeva 5, 1000 Ljubljana, Slovenia
[b] Australian Centre for Research on Separation Science (ACROSS), School of Chemistry, University of Tasmania, Private Bag 75, Hobart, Tasmania 7001, Australia

ARTICLE INFO

Article history:
Received 3 June 2013
Received in revised form 9 July 2013
Accepted 10 July 2013
Available online 13 July 2013

Keywords:
System peaks
Formation mechanism
Ion exclusion chromatography
Vacancy ion exclusion chromatography

ABSTRACT

Single or multiple system peaks can be observed in ion-exclusion chromatography (IEC) based on whether the eluent is composed of single or multiple active eluent constituents. It was confirmed experimentally that the number of system peaks is always equal to or lower than (when co-elution occurs) the number of active eluent components. Positive and negative system peaks can be recorded in the IEC systems. Negative system peaks can be expected for each eluent component that is also present in the injected sample plug at a lower concentration than in the eluent. In the opposite case, where the eluent components in the injected sample plug are present at a higher concentration than in the eluent, positive system peaks will be recorded. The retention times of individual system peaks are not dependent on each other, but rather depend on the capacity of the column toward the individual eluent component. This capacity depends on the nature of the eluent component and can be concentration-dependent (e.g. for 2,6-pyridinedicarboxylic acid) or concentration-independent (e.g. for acetic acid). The higher the column capacity, the longer the retention time of the corresponding system peak produced by that eluent component. The positive and negative system peaks are the result of column re-equilibration to an injected sample containing a higher or lower concentration of the eluent component, respectively. In general, positive system peaks have longer retention times than negative system peaks. The larger the concentration-dependent capacity of the IEC stationary phase toward an individual eluent component, the larger the retention time difference between positive and negative system peaks.

1. Introduction

Ion-exclusion chromatography (IEC) was first introduced by Wheaton and Bauman in 1953 [1]. IEC utilizes the interactions of charged analyte(s) with a stationary phase carrying functionalities with the same charge sign as the analytes. That is, anionic analytes are separated on cation-exchange materials bearing anionic functional groups (usually with an acidic eluent to regulate the charge on the analytes), and cationic analytes are separated on anion-exchange materials bearing cationic functional groups (usually with a basic eluent). One of the phenomena observed in IEC is the occurrence of single or multiple "system peaks", which may appear as positive or negative peaks, and which are not attributable to any of the analytes present in the sample, but rather to the eluent [2]. Tanaka et al. have described such system peaks as "eluent dips" on the basis that injection of water into an IEC system, equilibrated with a benzoic acid eluent, resulted in a negative system peak being observed at the retention time of benzoic acid [3]. O'Reilly et al. [4] attributed system peaks to a disturbance of eluent-stationary phase equilibriums, but the precise reason for this disturbance was not discussed in detail. In general, system peaks in IEC have been regarded only as a nuisance and for this reason there has been no detailed study of the mechanism by which they are formed. However, a new branch of IEC, termed "vacancy ion-exclusion chromatography (VIEC)", was developed by Tanaka et al. [5–8] and relies on the formation of "vacancy" peaks. VIEC is based on equilibration of the stationary phase (generally a cation-exchange material) with the sample (generally a mixture of weak organic acids) and a subsequent injection of pure water. Negative "vacancy" peaks appear in the resultant chromatogram, with the number of negative peaks being equal to the number of analytes present in the sample (unless co-elution of two or more analytes occurs).

The mechanism of retention in the IEC has traditionally been considered to be based on two main processes, namely the Donnan exclusion effect (involving repulsion of the charged analytes from the similarly charged functional groups on the stationary phase) and on the hydrophobic interactions between the analytes and the non-functionalized regions of the stationary phase [9]. However,

* Corresponding author. Tel.: +386 1 24 19 100; fax: +386 1 24 19 220.
E-mail addresses: milko.novic@fkkt.uni-lj.si, milko.novic@siol.net (M. Novič).

0021-9673/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.chroma.2013.07.041

these processes have not been used for a convincing account of the formation of system peaks in the IEC to date. We have proposed a more general separation mechanism for the IEC [10] which is based on two major competing processes, namely the diffusion of the analyte molecules into the electrostatic potential wells (or "pores") present on the stationary phase and on their Coulombic repulsion back into the eluent bulk. The intensity of the diffusion of the analytes into the pores is governed by the concentration gradient of the analyte, whereas the degree of the Coulombic repulsion of the analytes out of the pores is governed by the electrostatic potential in the pores and on the average charge of the analyte. Hydrophobic adsorption of the analytes onto the unfunctionalized regions of the stationary phase is not considered to play a significant role in the analyte retention [10]. A direct consequence of the species-dependent intensity of the Coulombic interactions occurring between the stationary phase and the charged eluent and analyte ions was found to be a strongly variable column capacity for these ions, dependent on the nature and concentration of individual species.

In the present paper, a detailed study of the mechanisms underlying the formation of the system peaks in the IEC and of the appearance of negative peaks in the VIEC is reported. As a basis for explaining these mechanisms, the following general observations on the behavior of the system peaks were noted. First, the number of system peaks is equal to or less than the number of eluent components (or sample components in the case of the VIEC). Second, the appearance of a system peak depends on the nature of the eluent component as well as on its concentration. Third, the negative system peaks usually exhibit tailing while the positive system peaks exhibit fronting. Fourth, the negative system peaks, appearing in the conventional IEC, are identical in nature to the "indirect" peaks observed in the VIEC.

2. Experimental

2.1. Materials and reagents

Water used for the experimental work was doubly distilled and then passed through a Milli-Q (Millipore, Bedford, MA, USA) water purification system. A stock solution of 0.1 M sulfuric acid was prepared by diluting 5.5 mL of 98% sulfuric acid (Ajax Chemicals, Australia) in Milli-Q water to 1 L in a volumetric flask. A stock solution of 0.1 M acetic acid (HAc) was prepared by diluting 5.7 mL of 99% acetic acid (BDH ANALAR, BDH Chemicals, Australia) in Milli-Q water to 1 L in a volumetric flask. 10 mM acetic acid was prepared by appropriate dilution of an aliquot of 0.1 M stock solution by Milli-Q water. A 20 mM solution of 2,6-pyridinedicarboxylic acid (PDCA) (Aldrich Chemical Company, Inc., USA) was prepared by dissolution of 3.34 g of PDCA in Milli-Q water and diluted to the mark in a 1 L volumetric flask.

2.2. Instrumentation

A Thermo Fisher Scientific (Sunnyvale, CA, USA) DX500 chromatography system was used, consisting of a GP40 quaternary gradient pump, an AS50 Autosampler, an AS50 chromatography compartment module and an ED40 conductivity detector module. The separation column was a Thermo Fisher Scientific ICE-AS1 column (9 mm × 250 mm, total capacity of 27 mequiv. of sulfonate groups), used in a Waters (Milford, MA, USA) TCM column temperature controller. When larger and variable volumes of Milli-Q water (system peak analysis) were to be used, the system described above was re-plumbed in such a way as shown in Fig. 1.

A Valco Instruments E60-230 (USA) valve was utilized, re-plumbed from the injector and controlled through relays 1 and 2

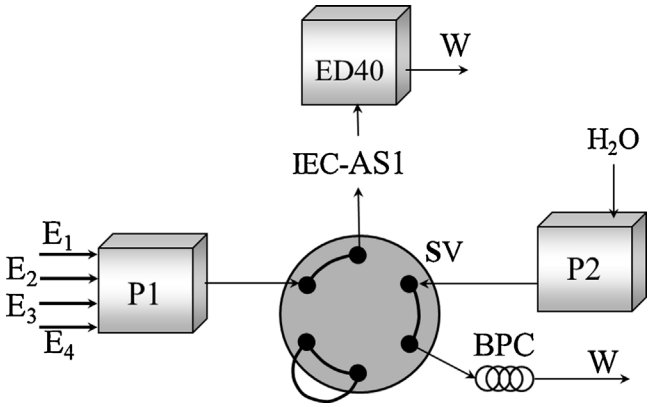

Fig. 1. IEC system used for the measurement of system peaks in ion exclusion chromatography. E1, E2, E3 and E4, selected eluents; P1, GP40 quaternary gradient pump (Thermo Fisher Scientific); P2, IP25 Isocratic pump (Thermo Fisher Scientific); SV, selection valve (re-plumbed E60-230 (Valco Instruments, USA)); BPC, back-pressure coil providing 850 psi of back-pressure at 1 mL/min flow-rate; IEC-AC1, ion exclusion column (IEC-AS1, Thermo Fisher Scientific); ED40, conductivity detector (Thermo Fisher Scientific); W, waste.

on the GP40 pump (Thermo Fisher Scientific). The variable sample loop volume was selected by changing the time the valve was in the position enabling the introduction of Milli-Q water. It was confirmed experimentally that the shortest reproducible injection time was 1 s, which corresponded to an injection volume of 16.7 μL. The eluent flow-rate was 1 mL/min in all experiments. The entire chromatography system was controlled using a Chromeleon 6.8 (Thermo Fisher Scientific) chromatography workstation. Further evaluation of data was also carried by application of Microcal Origin (Microcal Software, USA) to the exported data sets.

The system shown schematically in Fig. 1 enables complete automation of the measuring procedure. By appropriate pre-programming, the system can equilibrate the AS1 column with the selected eluent (E1-E4) or by a pre-programmed mixture of these four eluents. The injection volume was determined by the pre-programmed time interval and flow-rate when the pump P2 introduces pure water into the system. When positive system peaks were to be measured, the isocratic pump P2 was used to deliver the eluent at a pre-selected concentration to the analytical column, equilibrated with the same eluent carrying the same eluent component at a lower concentration than that in the injected sample plug. A back-pressure coil (BPC) was constructed of PEEK tubing to produce approx. 850 psi back-pressures at a flow-rate of 1.0 mL/min. This back-pressure was needed to prevent the pump from automatically switching off when the flow was redirected to waste.

3. Results and discussion

3.1. Column re-equilibration studies

The capacity of the IEC column toward a particular ion (analyte or eluent) can be measured using a breakthrough curve representing the column conversion from the selected eluent or analyte form into the water form (Fig. 2). In Fig. 2, the conversion of the AS1 column from the water form into the 2 mM acetic acid (HAc) or 2 mM pyridinedicarboxylic acid (PDCA) forms, and vice versa, is presented.

There are several important times marked on Fig. 2. The subscript A (see HAc_A and $PDCA_A$) marks the conclusion of the column conversion into the water form, while the subscript B (see HAc_B and $PDCA_B$) marks the time when the detector signal reaches a steady-state value in the column conversion from the water form into the selected eluent form (2 mM acetic acid or 2 mM PDCA). These two