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Application of retention modelling to the simulation of separation of organic anions in suppressed ion chromatography

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ABSTRACT

The ion-exchange separation of organic anions of varying molecular mass has been demonstrated using ion chromatography with isocratic, gradient and multi-step eluent profiles on commercially available columns with UV detection. A retention model derived previously for inorganic ions and based solely on electrostatic interactions between the analytes and the stationary phase was applied. This model was found to accurately describe the observed elution of all the anions under isocratic, gradient and multi-step eluent conditions. Hydrophobic interactions, although likely to be present to varying degrees, did not limit the applicability of the ion-exchange retention model. Various instrumental configurations were investigated to overcome problems associated with the use of organic modifiers in the eluent which caused compatibility issues with the electrolytically derived, and subsequently suppressed, eluent. The preferred configuration allowed the organic modifier stream to bypass the eluent generator, followed by subsequent mixing before entering the injection valve and column. Accurate elution prediction was achieved even when using 5-step eluent profiles with errors in retention time generally being less than 1% relative standard deviation (RSD) and all being less than 5% RSD. Peak widths for linear gradient separations were also modelled and showed good agreement with experimentally determined values.

1. Introduction

Chromatographic analysis plays a major role in the pharmaceutical industry and is applied throughout all stages of drug development, testing and production. Reversed-phase high performance liquid chromatography (RPLC) is by far the dominant separation mode used in these analyses because of its high efficiency and versatility. However, the separation selectivity attainable in RPLC is somewhat restricted and although there are some clear differences in selectivity between different reversed-phase (RP) materials, the broad elution sequence of analytes remains broadly constant over a wide range of RP stationary phases. Therefore, there is great interest in the pharmaceutical industry in the use of liquid phase separation systems which show separation selectivity that is strongly divergent from that of RPLC. Such systems might then be used to generate separations which complement RPLC, for example by providing data on impurities which cannot be separated by RPLC. Ion-exchange chromatography (IEC) offers considerable potential for the separation of ionic and ionogenic analytes and for such analytes it can usually provide separation selectivity which is complementary to RPLC. However, IEC has not found widespread routine usage in the pharmaceutical industry, largely because of the perceptions that it exhibits relatively low separation efficiencies and that method development is complex. For these reasons, ion-suppression and ion-interaction RPLC techniques are usually used for ionic and ionogenic analytes, rather than IEC.

IEC is a modern, high efficiency form of ion chromatography (IC) [1], which to date has been applied extensively to the separation of inorganic and small organic ions. The application of IEC to the separation of mixtures of larger, charged organic species, such as many ionogenic compounds of pharmaceutical importance, has also been demonstrated by several authors from as early as 1956 [2]. Subsequent work on IC of organic ions has focused on a range of small to medium sized organic anions including methanesulfonate [3], citrate [4], fluoroacetates [5–7], methylxanthine derivatives [8], carbocisteine [9], paracetamol and salicylic acid [10], as well as sodium cyclamate and other artificial sweeteners [11–16]. Although separation of the target compounds was achieved in all of these reports, detailed investigation into the effects on retention of competing ion type and concentration as well as the addition of organic modifier has been limited. Chen et al. investigated the separation of methylx-

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anthine [8] and several artificial sweeteners [15,16] and concluded from a qualitative investigation that retention was related to not only the charge on the organic species, but also to the concentration of competing ion and the percentage of organic modifier in the eluent. They attributed these dependencies to a mixed-mode retention mechanism involving both ion-exchange and hydrophobic interactions for organic anions.

The mechanisms underlying IC of inorganic ions are well established, with accurate models available to predict the retention behaviour of analytes and also to optimise the separation of mixtures of analytes by choice of the correct eluent composition [1,17–20]. Work within our laboratory has led to the development of retention models which accurately describe the retention characteristics of inorganic and low molecular mass organic anions and cations under isocratic and gradient conditions, as well as for complex elution profiles comprising multiple sequential isocratic and gradient steps [19,21]. In each case, the approach taken has been to perform a small number of experiments (typically 3) wherein retention is measured with eluents of known composition. These data are then used to derive parameters from the retention model and to then calculate retention of analytes over a designated search area of eluent compositions in order to simulate possible separations and identify the optimal eluent composition according to a desired optimisation criterion.

In the present study we extend this approach to the separation of larger organic anions (molecular mass up to 384) on conventional IC columns. The applicability of existing retention models (which consider only electrostatic interactions between analytes and stationary phase) is evaluated in order to elucidate the extent of mixed-mode retention involving both electrostatic and solvophobic effects. Finally, the prediction of retention for complex elution profiles comprising combinations of isocratic and gradient eluents formed from potassium hydroxide is assessed. These studies were aimed at determining the utility of ion-exchange separations in drug development and quality control in the pharmaceutical industry.

2. Experimental

2.1. Materials

All of the ionogenic organic compounds used as test analytes (see Table 1) were purchased from Sigma–Aldrich (Milwaukee, WI, USA). Sodium hydroxide (99.998%) was sourced from Sigma–Aldrich and chromatographic grade methanol was obtained from Merck (Darmstadt, Germany). Methanol was filtered through 0.22 μm nylon filters (Millipore, Bedford, MA, USA) before use. All other chemicals were used as supplied. Standard solutions of the test analytes were prepared directly in methanol and diluted into standard mixtures with further methanol. Potassium hydroxide eluents were prepared electrolytically using a Dionex Elu-Gen eluent generator, while sodium hydroxide eluents were prepared using 99.998% sodium hydroxide diluted in water purified using a MilliQ system (Millipore, Bedford, MA, USA). Eluents were filtered through 0.45 nylon filters (Millipore, Bedford, MA, USA) prior to use.

2.2. Chromatographic separations

A Dionex (Sunnyvale, CA, USA) ICS-3000 Ion Chromatography system consisting of a dual gradient pump unit (Dionex ICS-3000 DP), dual eluent generator unit (Dionex ICS-3000 EG), dual column and detector compartment (Dionex ICS-3000 DC), variable wavelength UV detector (Dionex ICS Series VWD) and autosampler (Dionex AS) was used for all separations. A Dionex GP40 gradient pump or an isocratic ICS-3000 DP dual pump was used to supply the external water supply to the suppressor. Eluent suppression was achieved using an ASRS ULTRAII (4 mm) electrolytic suppressor. All separations were performed using a 4 mm × 250 mm Dionex AS20 weak anion-exchange analytical column with a 4 mm × 50 mm Dionex AG20 guard column. Dionex AS16 (4 mm), AS11 (2 mm), AS11HC (2 mm), AS19 (2 mm) and AS24 (2 mm) analytical columns with their associated guard columns were used for comparative purposes, along with the Metrohm (Oberdorfstrasse, Herisau,

Table 1
Selected pharmaceutically relevant anionic compounds.

Analyte	Molecular mass	Log *P*	Relevant pK_a[a]		Charge at various pH (based on pK_a value[a])						
			pK_{a1}	pK_{a2}	pH 0	pH 1	pH 3.5	pH 7	pH 10.5	pH 13	pH 14
Phenol	94.11	1.49	9.86		0	0	0	0	−1	−1	−1
Valproic acid	144.2	2.75	4.82		0	0	0	−1	−1	−1	−1
Beta naphthol	144.2	2.8	9.57		0	0	0	0	−1	−1	−1
Tropic acid	166.2	0.28	3.85		0	0	0	−1	−1	−1	−1
1-Naphthoic acid	172.2	3.13	3.68		0	0	0	−1	−1	−1	−1
Acetylsalyclic acid	180.2	1.19	3.48		0	0	0	−1	−1	−1	−1
5-Methyl-5-phenylhydantoin	190.2	1	8.86		0	0	0	0	−1	−1	−1
Ibuprofen	206.3	3.5	4.41		0	0	0	−1	−1	−1	−1
Captopril	217.3	0.34	3.7	9.89	0	0	0	−1	−2	−2	−2
Naproxen	230.3	3	4.84		0	0	0	−1	−1	−1	−1
Mefenamic acid	241.3	5.33	3.73		0	0	0	−1	−1	−1	−1
Ketoprofen	254.3	3.12	4.23		0	0	0	−1	−1	−1	−1
Fenbufen	254.3	3.13	4.55		0	0	0	−1	−1	−1	−1
Sulindac	256.4	3.59	4.22		0	0	0	−1	−1	−1	−1
Tolfenamic acid	261.7	5.7	3.66		0	0	0	−1	−1	−1	−1
Flufenamic acid	281.2	5.25	3.67		0	0	0	−1	−1	−1	−1
Indoprofen	281.3	2.77	4.39		1	0	0	−1	−1	−1	−1
Chlorothiazide	295.7	−0.22	9.78		0	0	0	−1	−2	−2	−2
Diclofenac	296.2	4.06	4.18		0	0	0	−1	−1	−1	−1
Chloramphenicol	323.1	2.44	11.03	13.44	0	0	0	0	0	−1	−2
Furosemide	330.7	3	3.04	9.79	0	0	−1	−1	−2	−2	−2
Indomethacin (anionic)	357.8	4.27	3.96		0	0	0	−1	−1	−1	−1
Cortisone	360.4	1.44	12.4	12.95	0	0	0	0	0	−2	−2
Prednisolone	360.4	1.49	12.47	12.98	0	0	0	0	0	−2	−2
Hydrocortisone	362.5	3.5	12.48	12.98	0	0	0	0	0	−2	−2
Trichlormethiazide	380.7	0.24	7.12	9.51	0	0	0	0	−2	−2	−2
Althiazide	383.9	1.11	8.33	9.55	0	0	0	0	−2	−2	−2

[a] Calculated using ACD/Labs 7.00, Advanced Chemistry Development Inc., Toronto, Canada.