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Determination of pharmaceutically related compounds by suppressed ion chromatography: II. Interactions of analytes with the suppressor

Naama Karu[a], Greg W. Dicinoski[a], Melissa Hanna-Brown[b], Paul R. Haddad[a,*]

[a] Pfizer Analytical Research Centre, Australian Centre for Research on Separation Science, School of Chemistry, University of Tasmania, Private Bag 75, Hobart 7001, Australia
[b] Analytical R&D, Pfizer Global R&D, Ramsgate Road, Sandwich, Kent CT13 9NJ, UK

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ABSTRACT

For the hyphenation of ion chromatography to nebulising detectors or mass spectrometry, suppression of the non-volatile ionic eluent to water is a required step. However, suppression of weakly acidic or weakly basic organic analytes can potentially lead to losses of analytes during suppression resulting from precipitation, hydrophobic adsorption onto the suppressor, or permeation of the analyte through the suppressor membranes. This study investigates the interactions between the suppressor and weak organic acid analytes, including pharmaceutically related compounds, for eluents containing organic solvent. Correlations were observed between analyte recovery rates after electrolytic suppression and the eluent composition, the suppression conditions, and the physico-chemical properties of the analytes. These results suggest that hydrophobic adsorption interactions occur in the electrolytic suppressor and that these interactions are ameliorated by the addition to the eluent of high levels of organic solvents, especially acetonitrile. Use of eluents containing 80% acetonitrile resulted in very low losses of analyte during suppression. Recovery experiments conducted in various compartments of the electrolytic suppressor showed that some analytes permeated through the suppressor membrane into the regenerant chambers, but this could be prevented by adding organic solvent to the regenerant solution. It was also noted that analyte losses increased with ageing of the electrolytic suppressors. Chemical suppression avoids some of the analyte losses observed with an electrolytic suppressor, but when used under the correct conditions, electrolytic suppressors gave close to equivalent performance to chemical suppressors.

1. Introduction

Ion chromatography (IC) is a modern form of ion-exchange chromatography which uses high efficiency columns and electrogenerated eluents, typically coupled with conductivity detection, for the separation of small ions. IC is applied predominantly to the separation of inorganic anions and cations, but there is increasing interest in expanding the utility of IC to include organic ions, especially those of pharmaceutical interest. The prime motivation for this is that IC offers a separation mechanism and a separation selectivity which are complementary to those of reversed-phase high performance liquid chromatography (RP-HPLC). When applied to the task of identification of impurities in ionogenic pharmaceutical compounds, the use of both IC and RP-HPLC for the analysis will therefore increase the probability that minor impurities will be separated and quantified.

Hyphenation of IC to universal detectors, such as mass spectrometry or nebulising detectors (e.g. evaporative light-scattering detection) is also an attractive option to ensure that as many analytes as possible are detected. However, IC requires the use of an ionic eluent and such eluents typically cause severe interferences in these detectors. This difficulty may be overcome through the use of a suppressor situated between the column and the detector. IC suppressors are membrane-based devices which are designed to convert the ionic eluent to water as a means of enhancing the sensitivity of conductivity detection [1]. However, when used with universal detectors, the role of the suppressor is to act as a desalting device, thereby removing the interference resulting from the presence of ionic salts in the eluent. Suppressors are normally used with purely aqueous eluents, so there is a need to establish whether these suppressors can be used with the aqueous/organic eluents needed to elute organic analytes which are retained on the stationary phase through both Coulombic and hydrophobic interactions. Recently, we have shown that eluents using ionic gradients and containing organic solvents can be suppressed satisfactorily using either chemical suppression with a micromembrane suppressor or electrolytic suppression using a self-regenerating suppressor

* Corresponding author. Tel.: +61 3 62262179; fax: +61 3 62262858.
E-mail address: paul.haddad@utas.edu.au (P.R. Haddad).

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[2]. For utilisation in industry, the electrolytic suppressor is usually more appropriate since it can employ water as the suppressor regenerant and is fully automated in terms of response to changing eluent conditions. However, our previous study showed that care needed to be taken with controlling the suppressor current in order to avoid damage to the suppressor and also the generation of ionic components from oxidation of the organic solvent (especially methanol) present in the eluent.

Further potential problems arising when using suppressors as de-salting devices with organic analytes are the possibility of loss of analytes in the suppressor as a result of adsorption or precipitation effects, and dispersion of the analyte band in the suppressor. Weakly acidic analytes are anionic in the presence of the high pH eluents used with anion-exchange IC, but become protonated in the suppressor and are therefore prone to hydrophobic adsorption or precipitation. Similarly, weakly basic analytes are separated as cations with low pH eluents but are deprotonated in the suppressor to form neutral species. The micromembrane suppressors suppressor consists of layered ion-exchange membranes and fibrous chamber screens, with the regenerant chamber screen modified to possess a high ion-exchange capacity which serves as a reservoir for regenerant ions [3,4]. The suppressor membranes are constructed of PTFE, randomly grafted with styrene and functionalized with sulfone groups, leaving a heterogeneous structure with some hydrophobic pockets [5]. Hydrophobic interactions can occur between organic analytes (especially when they carry no charge) and the suppressor components, including adsorption and precipitation on the eluent screen or the hydrophobic pockets in the membrane. The high surface area of the suppressor membranes and screens translates into higher adsorptive capacity, potentially causing loss of analytes in the suppressor.

There is also a possibility of losses of analytes resulting from penetration of the analyte through the suppressor membrane into the regenerant chamber. Theoretically, anionic analytes are not able to penetrate the cation-exchange membranes of the anion suppressor due to the effects of Donnan exclusion [6]. However, Caliamanis et al. [7] observed loss of cyanide on an anion micromembrane suppressor using chemical suppression, which they related to the residence time of the analyte in the suppressor. Trans-membrane loss of organic acids through a home-made helical filament-filled membrane suppressor has also been reported by Okada and Dasgupta [8]. They found that the degree of loss depended on the pK_a of the analyte and the pH of the suppressed effluent. Although neither of these studies used electrolytic suppression or organic solvents in the eluent, the mechanisms referred to are still relevant to the present investigation. As part of the assessments of an electrolytic suppressor prototype, Strong and Dasgupta [9] examined the option that anionic analytes would be lost due to permeation through the membrane to the anode compartment. They compared the suppressed conductivity peak areas to those obtained using a packed-column suppressor but found no significant differences.

In principle, introduction of a suppression device between the column and the detector can be expected to cause some degree of peak broadening due to diffusional effects, which low suppressor volume can minimise [10]. The shape of the analyte band will also be influenced by hydrophobic adsorption effects, especially when the adsorption and desorption processes are slow. Examination of peak shapes and analyte losses can therefore provide important insight into the use of suppressors with organic analytes which are weakly acidic or weakly basic. It can be expected that peak area recovery rates after suppression are governed by a combination of hydrophobic interactions with the suppressor and permeation through the membranes, with the balance between these mechanisms being determined by eluent composition, suppression conditions and analyte properties.

In previous work [2] we have examined the efficiency of suppressors when operated with relatively high levels of organic solvents in the eluent. There have also been some previous studies showing mainly suppressed conductivity detection of aqueous/solvent eluents used for the determination of inorganic ions and small carboxylic acids [11–16] or long-chain sulfonic acids [17] and these studies have focused on separation aspects rather than the possible effects of the suppression step on analyte response. The present study concentrates predominantly on analyte losses during electrolytic suppression using acidic organic pharmaceuticals or surrogate compounds with molecular weight over 200 amu as analytes, with the suppressor operated in the external water mode for suppression of an ionic eluent prepared in an aqueous/organic solvent containing up to 40% (v/v) of organic modifier. Analyte losses during suppression are measured by comparison of UV absorbances at an isosbestic wavelength before and after suppression [8]. In addition, the presence of traces of analyte in the compartments of the suppressor is assessed, to further support mechanisms of interaction between the analytes and the suppressor. These studies are aimed ultimately to investigate the feasibility of linear response of a universal detector when coupled to an IC separation.

2. Experimental

2.1. Instrumentation

The IC system used in this study was a Dionex (Sunnyvale, CA, USA) ICS-3000 instrument, consisting of a dual gradient pump unit (DP), dual eluent generator unit (EG), autosampler (AS) and dual column and detector compartment (DC). The system setup was as described previously [2]. Organic solvents used in the eluents were introduced after the EG [18] through a 3-port tee-piece connector (Upchurch Scientific, Oak Harbor, WA, USA), using an additional HPLC pump (Jasco PU-2089i, Easton, MD, USA, or Dionex Ultimate 3000 gradient pump), followed by a gradient mixer (Dionex GM-3 4 mm). An additional pump (Varian 9010; Varian Associates, Tokyo, Japan) was used to provide water at 1 ml/min to the continuously regenerated trap column (CR-TC) and degasser, due to leaching of organic solvent through the suppressor membrane into the regenerant effluent which is normally used to provide water to the CR-TC and degasser. In the case of chemical suppression, a Dionex anion micromembrane suppressor (AMMS-300) was used and the regenerant (delivered through one of the DP pumps) was 15 mM sulfuric acid at a flow rate of 3.0 ml/min. Electrolytic suppression was performed using a Dionex anion self-regenerating suppressor (ASRS-300) operated at ambient temperature in an external water mode with a 3.0–5.0 ml/min regenerant flow-rate (delivered through one of the DP pumps). When aqueous eluent was in use, the setup was kept identical, introducing water through the tee-piece instead of organic solvent. The conductivity detector located in the DC was corrected to 35 °C with a temperature coefficient of 1.7%. Direct spectrometry was conducted using a Waters 486 single wavelength absorbance detector (Waters Corporation, Milford, MA, USA) located either before or after the suppressor. The UV detector was connected via a Dionex UI-20 universal interface to the Chromeleon (Version 6.80) data acquisition system. The isosbestic UV absorbance wavelengths were chosen for each analyte by an offline spectrophotometric assay performed on a Metertech UV/VIS SP 8001 (Metertech Inc., Taipei, Taiwan). Samples containing acetonitrile were kept in glass vials (Dionex 2.0 ml for matching AS tray) since standard 10 ml plastic vials were found to be non-compatible with high concentrations of acetonitrile.

Peak area recovery assays were performed on a Dionex anion-exchange guard column IonPac AG-11 (50 mm × 4 mm), with or without an analytical column AS-11 (250 mm × 4 mm) or on AS-11

HC or AS-20 (250 mm × 4 mm) anion exchange columns equipped with the matching guard column and heated to 30 °C.

For the determination of analyte traces in the suppressor compartments, an IonPac AS-20 (250 mm × 4 mm) analytical column was used, and the collected regenerant was concentrated using a Dionex On-Guard II RP trap column. Concentrated fractions were separated on an RP-HPLC system Waters 2695 equipped with Waters 996 PDA detector and a Dionex Acclaim Polar advantage C16 column, 250 mm × 4.6 mm. HPLC data acquisition was conducted on Empower pro v.5.

Physico-chemical properties of the test analytes were obtained using ACDLabs software v.12.0 (Advanced Chemistry Development Inc., Toronto, Canada). Based on the molecular structure of the analytes, this software predicted log *P* (Partition coefficient), log *D* (Distribution coefficient) at pH 5.2–7.0, pK_a and polar surface area (PSA, the sum of surface over all polar atoms in a molecule). Statistical analysis of the results was performed on Excel™ (Microsoft Corporation, Redmond, Washington, USA), using the analysis ToolPak add-in.

2.2. Materials

Aqueous solutions were made with Ultra-pure 18.2 MΩ Milli-Q water filtered through a 0.20 μm filter (Millipore, Bedford, MA, USA). Potassium hydroxide solutions were prepared from semiconductor-grade pellets (Sigma–Aldrich, Milwaukee, WI, USA). Hydrochloric acid solution was prepared by dilution of concentrated hydrochloric acid of analytical grade (Ajax Finechem, Australia). HPLC gradient grade methanol was obtained from Merck (Darmstadt, Germany) and acetonitrile (HPLC 190 nm grade) from Ajax Finechem (Australia). Solvents were filtered through Millipore 0.22 μm nylon filters and degassed before use. Phosphate buffer (pH 2.9) for RP-HPLC was prepared using phosphoric acid AR (Ajax Finechem, Australia), filtered, diluted and degassed. All other chemicals were used without further purification. Benzoic acid, sodium benzoate and trans-cinnamic acid were purchased from BDH Chemicals (Poole, Dorset, UK). Phenylacetic acid was sourced from Hopkin & Williams (Essex, UK). Benzenesulfonic acid was acquired from TCI (Fukaya, Japan). Ibuprofen, mefenamic acid, naproxen, sulindac, 1-naphthoic acid, 2-phenylbutyric acid, 2-phenylsuccinic acid, 4-heptylbenzoic acid and sodium 4-octylbenzenesulfonate were purchased from Sigma–Aldrich. A 0.1 M solution of hexanesulfonic acid was pre-mixed by Dionex (Sunnyvale, CA, USA). Standard solutions were prepared in the appropriate aqueous-organic solvent to give the required matrix composition. For spectroscopy measurements, 100 ppm standard solutions of the organic pharmaceutical analytes were used, with the solution also containing 1–4 mM potassium hydroxide when the analyte was in the salt form, and water or 1 mM hydrochloric acid for the acid form of the analyte. Potassium hydroxide eluent was prepared electrolytically using a Dionex EluGen eluent generator equipped with potassium hydroxide cartridge. Sulfuric acid regenerant (15–50 mM) was prepared daily from a 1 M stock solution made by dilution of 98% sulfuric acid (Ajax Finechem).

2.3. Procedures

2.3.1. Selection of isosbestic wavelengths

The isosbestic point is a wavelength in which the salt and free acid forms of the compound have the same molar absorptivity. Selection of UV absorbance wavelength for each analyte was carried out by comparison of the full absorbance spectra for the salt and acid forms of the analyte in the non-suppressed and suppressed eluent matrices. Depending on the desired form of the analytes, either acid or base was added to an aqueous/organic solvent solution containing the analyte. In cases where more than one isosbestic point was observed, the highest wavelength was chosen to prevent interference from the organic solvent, provided that the absorptivity was of a satisfactory level. If an analyte in a specific eluent composition did not show a good isosbestic point, it was omitted from tests under those conditions. Where applicable, the results obtained from two isosbestic wavelength were compared to verify the method accuracy.

2.3.2. Chromatographic conditions

Isocratic runs were performed using a narrow range of eluent concentrations in order to minimise differences in suppression between analytes. The separation was therefore adjusted by selection of different columns to suit the analyte retention profile under the selected conditions. The UV absorbance at the selected isosbestic point wavelength for each analyte was compared before and after suppression. All experimental points were performed at least in triplicate for each sample concentration. A total of 4 ASRS units and 2 AMMS units were examined.

2.3.3. Analyte traces in electrolytic suppressor

Analyte loading and recovery assays were performed for assessment of suppression by an ASRS electrolytic suppressor only. 100 μl of 1 mM ibuprofen in acetonitrile was separated on a single AS-20 column, two or three columns in series, under isocratic conditions of 6 mM KOH in 40%, 60% or 80% acetonitrile, respectively. The regenerant was collected for 5 min starting at the point of peak elution, and the suppressor chambers were then washed by 10 ml acetonitrile following the run. The collected acetontrile solution was then concentrated by evaporation and a 10 μl sample injected onto a reversed phase column operated with a 50% acetonitrile in phosphate buffer pH 2.9 eluent at a flow-rate of 1 ml/min. The column oven was set to 35 °C and the sample compartment set to 25 °C. Ibuprofen was well-resolved from other compounds and was detected using a photodiode array detector. All runs were conducted in triplicate and quantification obtained from linear regression equation of calibration plots with correlation coefficient ≥0.9999.

3. Results and discussion

3.1. Analyte test set

Hyphenation of suppressed IC to universal detectors aims predominantly at the analysis of non-chromophoric compounds. However, to address the objective of the current study, only UV absorbing analytes were selected so that UV absorbance measurements could be used to evaluate losses of analytes in the suppressor. The analyte test set consisted of 13 organic acids of general pharmaceutical relevance, chosen to reflect a range of physico-chemical properties of potential relevance to possible interactions with the suppressor, with variations in structure such as side chains, charge, or number of aromatic rings (Fig. 1, Table 1). Not all analytes were tested under all experimental conditions as some of the more hydrophobic compounds exhibited solubility problems and were only tested in eluents containing sufficient amounts of organic solvent. Analytes which exhibited full recovery were not used in subsequent assessment stages.

3.2. Effect of eluent matrix on analyte recovery

Analyte recovery rates were measured after suppression in an aqueous eluent, followed by eluents containing up to 40% (v/v) methanol or acetonitrile. Recoveries were assessed by UV absorbance measurements conducted at an isosbestic point wavelength between the ionised and neutral forms of the analyte, so that there would be no differences in absorptivity between these two

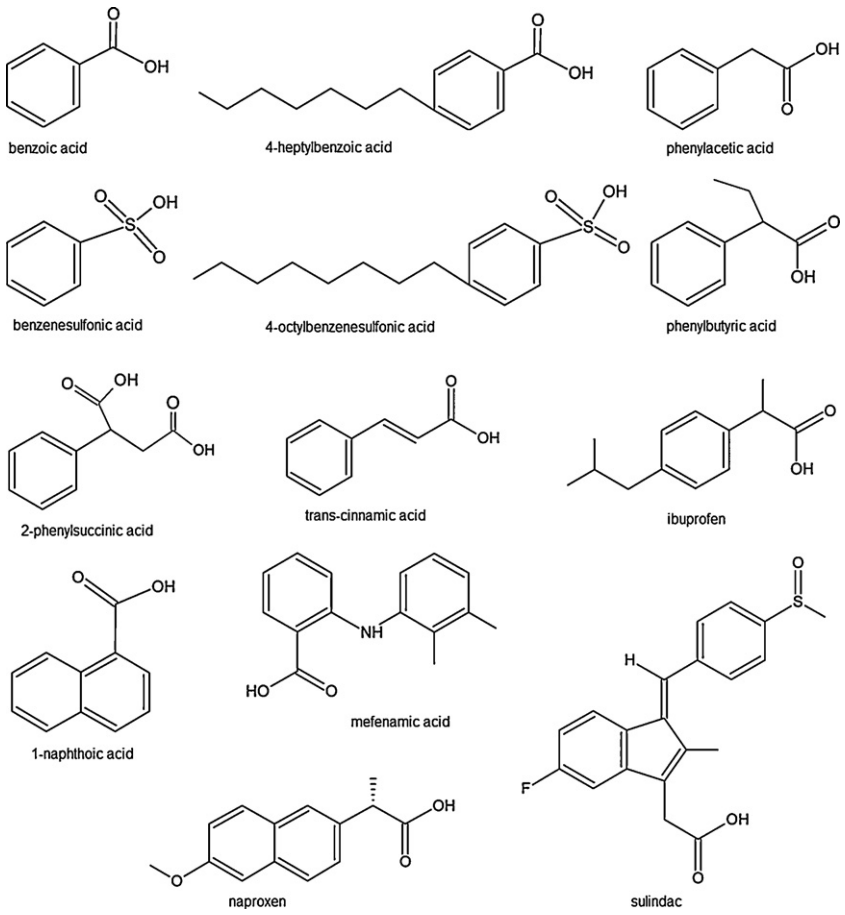

Fig. 1. The molecular structures of the analytes used in this work.

forms of the analyte. In this way, the degree of protonation of the analyte during suppression (governed by the pK_a of the analyte and the pH of the suppressed eluent) did not influence the calculation of the recovery. Recovery values are given in Table 2, which shows that four of the test analytes gave 100% recovery after suppression in an aqueous eluent, with three further analytes showing substantial losses and the remainder being insoluble in the aqueous eluent at the concentration evaluated.

The two analytes (trans-cinnamic acid and ibuprofen) exhibiting recovery rates below 80% in the aqueous eluent showed significantly improved recovery rates when the eluent contained increasing concentrations of methanol, yet full recovery was not attained for any of the weak acids (Table 2). The strong influence of analyte charge is demonstrated by comparing 4-octylbenzenesulfonate and 4-heptylbenzoic acid. Both have relatively high log *P* values (Table 1), but the former is much more acidic than the latter and exists completely in its anionic form at the pH of the suppressed eluent (pH 5.2). This charge is sufficient to prevent adsorption of 4-octylbenzenesulfonate onto the suppressor, unlike 4-heptylbenzoic acid which has a pK_a of 4.36 and is hence

Table 1
Test set of organic acid analytes. All data calculated by ACD/Labs, or taken from references included in ACD/Labs 12.00 (Advanced Chemistry Development Inc., Toronto, Canada) unless mentioned otherwise.

Analyte	MW (g/mol)	Log *P*	Relevant pK_a	Log *D* at pH			Polar surface area (Å^2)
				5.2	5.8	7.0	
Benzoic acid	122.1	1.89	4.20	0.86	0.30	−0.74	37.3
Benzenesulfonate	158.2	0.47	0.70	−3.03	−3.03	−3.03	62.8
Phenylacetic acid	136.2	1.50	4.25[22]	0.55	−0.01	−1.16	37.3
2-Phenylbutyric acid	164.2	2.38	4.34	1.47	0.91	−0.24	37.3
2-Phenylsuccinic acid	194.2	1.22	4.29[a]	−0.82	−1.68	−3.42	74.6
Trans-cinnamic acid	148.2	2.41	4.45	1.07	0.49	−0.62	37.3
4-Octylbenzenesulfonate	294.4	4.65	−0.44	1.15	1.15	1.15	62.8
4-Heptylbenzoic acid	220.3	5.54	4.36	4.64	4.09	3.02	37.3
1-Naphthoic acid	172.2	3.13	3.69	1.60	1.04	0.20	37.3
Ibuprofen	206.3	3.72	4.41[b]	2.87	2.32	1.16	37.3
Naproxen	230.3	3.00	4.84	2.48	2.00	0.85	46.5
Mefenamic acid	241.3	5.33	4.20[22]	3.85	3.29	2.42	49.3
Sulindac	354.0	3.59	4.22	2.57	2.00	0.85	54.4

[a] Value is average of pK_{a1} and pK_{a2}.
[b] Reference pK_a Values for ibuprofen are between 4.4 and 5.3 [23].

Table 2
Highest percentage peak area recovery rates obtained after suppression by ASRS-300 (used or new unit as indicated). The suppressed eluent was aqueous KOH, or KOH in 25% or 40% (v/v) methanol or acetonitrile. The percentage recovery error is one standard deviation ($n = 3$).

Analyte	Aqueous KOH	KOH in methanol			KOH in acetonitrile	
		25% Used	40% Used	40% New	40% Used	40% New
Benzoic acid	100.1 ± 0.9					
Benzenesulfonate	100.1 ± 1.0					
Phenylacetic acid	100.6 ± 0.9					
2-Phenylbutyric acid	100.3 ± 1.4					
2-Phenylsuccinic acid	89.1 ± 0.5					
Trans-cinnamic acid	76.4 ± 0.6	97.0 ± 1.0	98.0 ± 0.3			
4-Octylbenzenesulfonate	n/s	100.7 ± 0.3	101.0 ± 0.3			
4-Heptylbenzoic acid	n/s	n/s	27.6 ± 0.6	54.7 ± 0.8	59.7 ± 0.5	75.9 ± 0.5
1-Naphthoic acid	n/s	82.6 ± 0.6	86.7 ± 0.8	92.2 ± 1.0	74.9 ± 0.1	89.8 ± 0.2
Ibuprofen	40.0 ± 0.4	66.5 ± 0.4	78.3 ± 1.4	89.3 ± 1.5	65.5 ± 0.7	83.7 ± 0.5
Naproxen	n/s	n/s	85.3 ± 0.1	87.9 ± 1.8	75.7 ± 0.2	95.0 ± 1.0
Mefenamic acid	n/s	40.3 ± 0.7	57.3 ± 1.7	59.8 ± 0.3	81.5 ± 1.0	91.6 ± 0.3
Sulindac				90.1 ± 0.6		96.2 ± 2.0

n/s: 500 ppm not readily soluble in the matrix or isosbestic point could not be established.

only partially charged and therefore exhibits substantial losses in the suppressor, even when the eluent contained 40% methanol. This analyte also exhibited extensive peak broadening and peak tailing after passing through the suppressor.

One of the obstacles in assessing electrolytic suppressors is the fragility of the membranes and screens, which exhibit deterioration over time under harsh conditions, such as the use of high concentrations of organic solvents combined with high electrical currents [3,5,12]. When a used suppressor unit was replaced by a new one, the recovery rates improved for some of the analytes (Table 2). This trend can be explained by increased hydrophobicity of an aged membrane as a result of loss of ion-exchange functionality. In addition, permeation of analytes through an aged membrane is possibly increased due to a reduction in the Donnan exclusion effect. However, persistent low recovery rates regardless of the age of the ASRS were observed for mefenamic acid and 4-heptylbenzoate. These analytes have the highest log *D* and log *P* values in the test set and are therefore more prone to precipitate or adsorb upon protonation.

The addition of acetonitrile to the eluent further improved the solubility of the analytes and reduced hydrophobic interactions in the suppressor, leading to improved recoveries in some cases, especially for analytes with high log *P* and log *D* values. Switching to a new suppressor unit again resulted in a further increase in recovery rates for all analytes.

3.3. Effect of applied current and measured voltage

Application of an unnecessary high current is not uncommon when suppressing a steep ionic gradient, if applying a constant current and not a gradient of currents [2] or current switching [19]. The applied current level is dictated by the highest eluent concentration of the gradient hence at the low range of ionic concentration of the gradient, excess current is applied, producing hydronium at a higher rate than required for neutralising the eluent, and also generating more waste gases, causing baseline noise and oxidation effects [20]. Dimitrakopoulos and co-authors examined the effect of this scenario on peak shape and recovery of amphoteric herbicides separated by a gradient of aqueous eluent [21]. Based on the suppressed conductivity signal, they reported that application of higher currents caused peak tailing and a decrease of 3–7% in peak area compared to the ideal low current. In the present study, the effect of suppression current on the recovery of ibuprofen was assessed using a constant eluent KOH concentration in 40% (v/v) acetonitrile, while varying the current from the recommended level up to a high current typical for a gradient run. Fig. 2(a) shows that elevation of the current level caused increased band broadening and asymmetry. Although governed primarily by the input current, the voltage may vary for a constant current since the resistance in the suppressor depends on its usage, and also the presence of contaminants and organic solvents [3]. Fig. 2(b) shows that band broadening, and to a lesser extent also band asymmetry, increased with higher suppression voltages. As demonstrated for mefenamic acid in 30 mM KOH containing 40% acetonitrile (Fig. 3), the voltage levels recorded over several months of operation increased while recoveries decreased. High voltage can be a symptom of suppressor deterioration which is accompanied by a higher rate of hydrophobic interactions, or can directly enhance analyte transport due to over-heating and induced diffusion across the membranes [5,9].

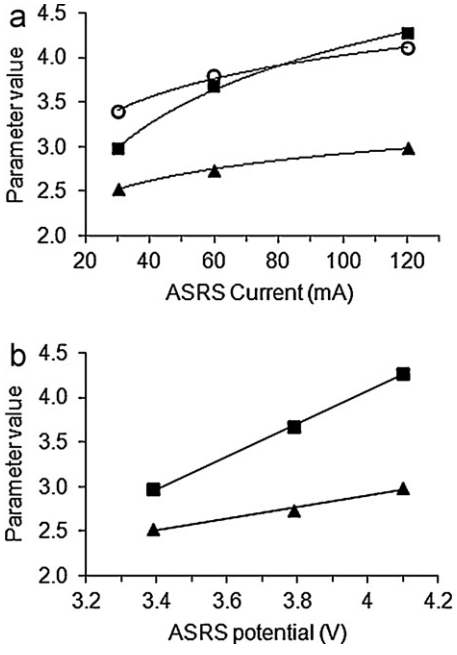

Fig. 2. Effects of suppressor current and voltage on peak shape and recovery rate of 25 μl 100 μM ibuprofen separated on an AS-11 HC column by 12 mM KOH eluent containing 40% ACN. (a) Measured potential on a used ASRS (open circles); suppressed peak asymmetry at 5% peak height (filled triangles); band broadening (filled squares) is the ratio between peak width after and before suppression (at 4.4% peak height). (b) Correlation between the suppressor voltage in (a) and band broadening (filled squares) and asymmetry (filled triangles).

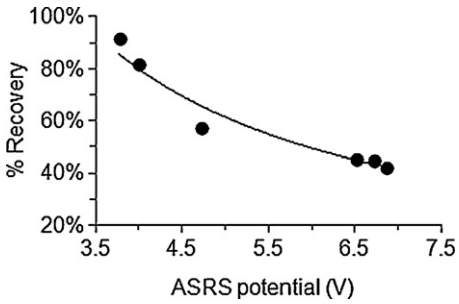

Fig. 3. The relationship between voltage levels measured on a used ASRS and peak area recovery of 25 μl 100 μM mefenamic acid after suppression of 30 mM KOH in 40% ACN at several time points along a year.

3.4. Measurement of analyte in the suppressor compartments

In an attempt to elucidate the sources of analyte loss during suppression by the ASRS, measurements of analyte concentrations in the various compartments of the suppressor were undertaken. Adsorbed or precipitated analyte should be extractable from the eluent chamber by washing this chamber with organic solvent. Alternatively, analyte which has penetrated through the suppression membrane to the regenerant chamber can be collected in the regenerant stream or it can be extracted from the regenerant chamber. The analyte chosen for this investigation was ibuprofen, which exhibited good solubility in aqueous/organic eluents, yet displayed a persistent low recovery under diverse suppression conditions. In order to minimise solubility issues, at least 40% (v/v) acetonitrile was added to the eluent for this study. Under these conditions, ibuprofen can be separated on IC columns using very low concentrations of ionic eluent, hence suppression can be accomplished by application of low levels of current (15 mA), thereby reducing any potential analyte losses due to current effects. Under the HPLC conditions used to measure the levels of ibuprofen in the suppressor compartments, the limit of detection (LOD) for ibuprofen was 0.25 μM (2.5 pmol on column) based on signal to noise ratio of 3. This LOD was sufficient to quantify less than 0.01% of the 100 nmol of ibuprofen injected onto the IC for each recovery experiment.

The results of the recovery experiments are shown in Fig. 4. On a used ASRS applied to suppression of an eluent containing 40% (v/v) acetonitrile (Fig. 4(a)), the recovery rate for ibuprofen was 61.0%, with less than 3% of the injected ibuprofen being recovered from the regenerant chamber, and 17% recovered from the eluent chamber. A remaining 19.5% of the injected ibuprofen could not be identified. Next, eluent containing higher concentrations of acetonitrile was used, with the aim of reducing hydrophobic interactions between the analyte and the membranes and screen of the eluent chamber. Fig. 4(b) illustrates how increasing the acetonitrile content in the eluent to 60% improved the recovery rate to 75.0% and led to reduced ibuprofen detected in the eluent chamber. Further increasing the acetonitrile level in the eluent to 80% (Fig. 4(c)) raised the recovery rate to 92.5%, but also increased the percentage of the ibuprofen found in the regenerant chamber. This indicated that permeation of ibuprofen through the suppressor membrane was enhanced in the presence of higher levels of acetonitrile. It is interesting to see that the values extracted from Fig. 4 shows a linear trend with the percentage of acetonitrile added to the eluent (Fig. 5).

The exposure of the ASRS to acetonitrile concentrations higher than 40% while applying current is considered to be potentially harmful to the suppressor [3,5]. To evaluate this aspect, the experiments with eluent containing 40% (v/v) acetonitrile shown in Fig. 4(a) were repeated at the conclusion of the multiple assays undertaken with 60 and 80% (v/v) acetonitrile eluent shown in

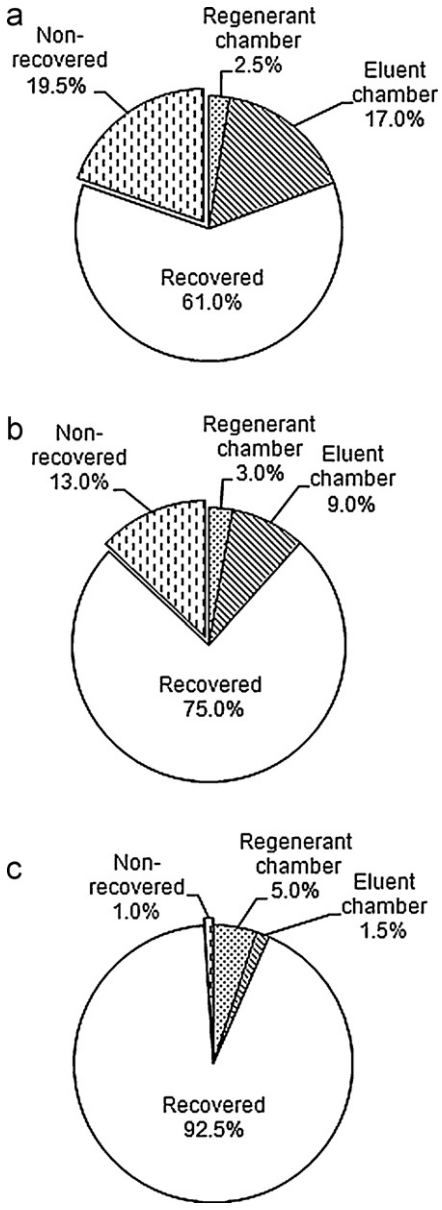

Fig. 4. Distribution between suppressor compartments of ibuprofen after suppression. Experiments involved (a) suppression of KOH in 40% ACN, (b) suppression of KOH in 60% ACN or (c) suppression of KOH in 80% ACN.

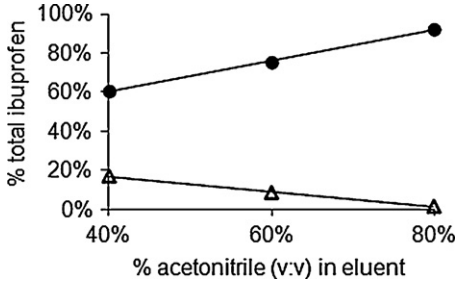

Fig. 5. The effect of acetonitrile content in the eluent on the recovery of ibuprofen after suppression (filled circles) and the ibuprofen extracted from the suppressor eluent chamber (open triangles), as shown in Fig. 4(a)–(c).

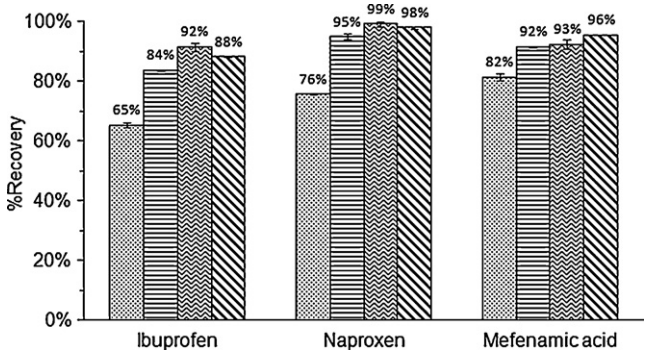

Fig. 6. Recoveries for ibuprofen, naproxen and mefenamic acid after suppression by (from left to right) a used ASRS (dotted), new ASRS (horizontal lines), new ASRS using 40% ACN regenerant (zig zag lines) and by AMMS (diagonal lines). The recovery rates are shown as percentages above each column, and the error bars are one standard deviation based on $n \geq 3$. The eluent was KOH in 40% ACN, 20 mM for naproxen and ibuprofen and 30 mM for mefenamic acid. All separations were on an AS-11 HC column.

Fig. 4(b) and (c). The recovery rate dropped from 61.0% to 50.4%, and the increased loss was distributed between the regenerant and eluent chambers of the suppressor. This suggested that the continuous application of organic solvent had similar effects on both the penetration and adsorption mechanisms of analyte loss.

3.5. Comparison with other suppression methods

A comparison of the ASRS used in the electrolytic mode with chemical suppression using the AMMS was undertaken using mefanamic acid, naproxen and ibuprofen as analytes. The assays were conducted only for eluent containing 40% acetonitrile, which was considered more relevant for future work, based on the results gathered so far. Since a chemical suppressor does not utilise an electric field, it is expected to suffer less degradation over time compared to an electrolytic suppressor, as well as to avoid other implications caused by current. Therefore, apart from offering another option for suppression, chemical suppression can assist in isolating the factors causing analyte loss in electrolytic suppression. A comparison is not straightforward since on a chemical suppressor the ion-exchange is performed on the membranes on both sides of the eluent chamber, while electrolytic suppression enables ion-exchange only on one membrane (on the cathode side) [9]. As a further facet of investigation arising from the detection of traces of analyte in the regenerant chamber of the suppressor, 40% (v/v) of acetonitrile was added to the regenerant of an electrolytic suppressor in order to examine whether this would influence passage of the analytes through the suppressor membrane.

Fig. 6 shows the recovery rates for each of the tested analytes on a range of suppressors. In general, the used ASRS performed worst, with lowest recoveries and highest band broadening and peak asymmetry. A new ASRS unit was significantly better, although generally optimal performance was exhibited either with chemical suppression on AMMS or electrolytic suppression with 40% acetonitrile added to the regenerant. It is noteworthy that continued use of regenerant containing such high concentrations of organic solvent may be likely to permanently damage the suppressor [5].

3.6. Hydrophobic interactions between suppressor and analyte

Low analyte recovery observed on electrolytic suppressors can be attributed primarily to hydrophobic interactions between the analyte and the suppressor. As mentioned earlier, exposure of the electrolytic suppressor to high currents, especially in the presence of high concentrations of organic solvents, can decrease the ion-exchange capacity of the membranes and damage the screens. Such damage is also observed by high voltage levels across the suppressor after long periods of usage. An increase in the hydrophobic area of the suppressor available for interaction with the analytes should result in some correlation between recovery rates and peak dispersion with log D (or log P) of the analytes. Fig. 7(a) shows the relationship between log D and recovery rate for a used ASRS with eluents containing 25% or 40% methanol, illustrating how increased log D of the analytes leads to decreased recovery. For eluents containing acetonitrile, no clear relationship between log D and recovery rate was observed, reflecting the much high eluotropic strength of acetonitrile compared to methanol.

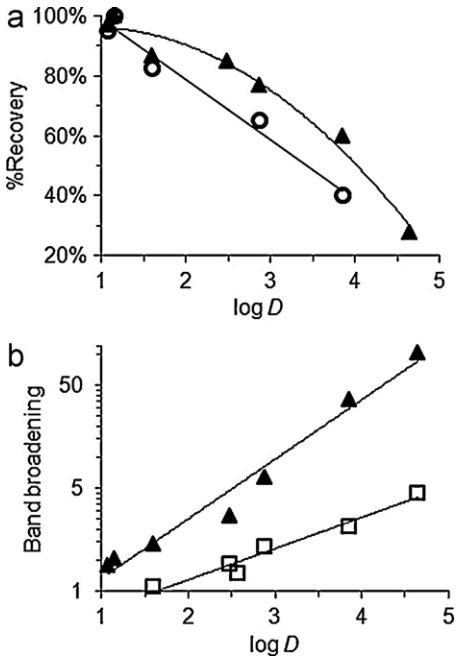

Fig. 7. (a) Analyte recovery rate as a function of log D for ASRS suppressing KOH in 25% MeOH (open circles) or in 40% MeOH (filled triangles). (b) Band broadening as a function of log D after suppression of KOH containing 40% MeOH (filled triangles) open squares on a logarithmic scale. Band broadening is normalised: (non-suppressed peak width at 4.4% height)^2/suppressed peak width.

The relationships between band broadening and log D of the analytes for an ASRS suppressor employed with eluents containing either methanol or acetonitrile are shown in Fig. 7(b). This figure shows that band broadening increased with increasing log D, with eluents containing methanol showing greater effects than those containing acetonitrile. Generally, a used suppressor showed greater band broadening than a new suppressor (data not shown). These results suggest that acetonitrile can reduce hydrophobic interactions between the analytes and the suppressor more effectively than methanol. As shown earlier, analyte losses in the suppressor can be eliminated almost entirely by addition of very high concentrations of acetonitrile to the ionic eluent. However, this approach is far from ideal for the suppressor itself, exposing it to harmful conditions which induce deterioration of the membranes and screens. Furthermore, the chosen eluent composition should be governed primarily by the desired selectivity of the separation step rather than the suppression step. In this regard, eluents containing acetonitrile concentrations higher than 40% (v/v) substantially reduced the retention of some target analytes on the high-capacity anion exchange columns used, thereby making it more difficult to achieve the required separation.

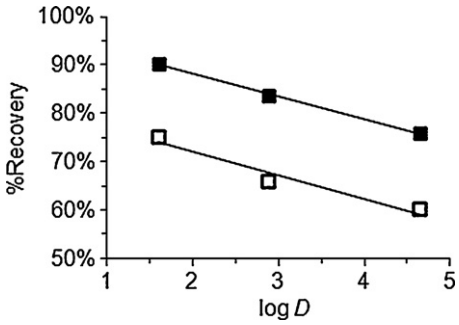

Fig. 8. Recovery rate of analytes with PSA <40 as a function of log *D* at pH 5.2. Used ASRS (open squares) compared to a new ASRS (filled squares) suppressing 20 mM KOH containing 40% ACN log D was calculated at a pH of 5.2.

3.7. Permeation of analyte through the suppressor membranes

The experiments described in Section 3.4 confirmed that permeation of ibuprofen through the suppressor membrane had occurred. As mentioned previously, as a weak acid passes through the eluent channel in the suppressor, it becomes protonated and is therefore more likely to permeate through the membrane in its uncharged form. In 40% acetonitrile, the recovery rates of the analytes could be classified into two groups according to the molecular weight of the analyte (above or below 225 g/mol) or according to the polar surface area (PSA) of the analyte (above or below 40 $Å^2$). PSA is commonly used as a parameter to predict permeation of pharmaceuticals through membranes. Analytes with higher molecular weight and higher PSA showed increased and relatively consistent recovery rates along a wide range of log *P* or log *D*. Conversely, as Fig. 8 shows, analytes having lower molecular weight and low PSA values (<40) exhibited recovery rates which decreased with log *D*, with the observed recovery rates being dependent on the age of the suppressor. Lower molecular weight may suggest easier transport through the suppressor membranes, which is also facilitated by lower polar surface area. Penetration of analytes into the regenerant chamber was exacerbated for eluents containing significant levels of organic solvents due to the flux of organic solvent from the eluent chamber into the regenerant chamber. This flux can be eliminated by adding organic solvent to the regenerant and under these conditions the observed change in recovery rate was strongly correlated to the pK_a and inversely correlated to the PSA of the analytes. However, addition of organic solvent to the regenerant is not a recommended long-term solution to permeation of the analytes through the membrane because this practice is likely to cause deterioration of the membranes and the regenerant screens [5].

Finally, a further potential cause of analyte permeation through the suppressor membrane is electrophoretic movement of the analytes under the influence of the electric field in the suppressor. However, this would be relevant only for analytes which remain ionic in the suppressor and would be unlikely at the low voltages used.

4. Conclusions

This study has examined the interactions occurring between weak organic acid analytes and membrane suppressors, using eluents containing organic solvents. Such eluents are essential for improving peak shape and for manipulating retention when these analytes are chromatographed on polymeric ion-exchange columns. The addition of organic solvents, especially acetonitrile, improved the solubility of the more hydrophobic analytes which were prone to precipitation in the suppressor and also aided in minimising adsorption of the analytes on the suppressor screens and membranes. Such hydrophobic interactions between the analytes and the suppressor are the largest source of low recovery rates for analytes following suppression. On the other hand, organic solvents may damage the suppressor over time and induce some permeation of the analytes through the suppressor membranes. Nevertheless, high recovery rates for hydrophobic analytes can only be achieved if relatively high levels of acetonitrile are present in the eluent.

The problems encountered with non-quantitative analyte recoveries when using electrolytic suppressors could be solved partially by switching to chemical suppression, although this type of suppression is also more expensive to run. Alternatively, high analyte recoveries could be achieved using electrolytic suppressors when the eluent contained high levels (e.g. 80%) of acetonitrile, although this in turn substantially reduced the retention of analytes on even the highest-capacity anion exchange columns. Finally, it was noted that analyte losses during electrolytic suppression were increased as the suppressor aged, presumably due to loss of ion-exchange functionalities from the suppressor screens and membranes.

In view of the results obtained, a redesigned suppressor that is more compatible with organic solvents and minimises hydrophobic interactions with analytes would be desirable. Prototypes of new electrolytic suppressors are currently under examination and their performance will be described in a subsequent report.

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