Journal of Chromatography A, 1233 (2012) 71–77

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

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

Determination of pharmaceutically related compounds by suppressed ion chromatography: III. Role of electrolytic suppressor design

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ARTICLE INFO

Article history:
Received 20 December 2011
Received in revised form 31 January 2012
Accepted 2 February 2012
Available online 11 February 2012

Keywords:
Ion chromatography
Suppressor designs
Pharmaceuticals

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 to avoid elevated detector baselines. Presented here is a study of three new designs of electrolytic suppressors, incorporating high ion-exchange capacity screens and high ion-exchange capacity membranes in different thickness and compositions. These designs aim to minimise hydrophobic interactions of the suppressor with organic analytes and to provide higher compatibility with eluents containing acetonitrile. In comparison with a commercially available electrolytic suppressor and also a commercially available chemical suppressor, the new high-capacity suppressor showed superior performance, exhibiting minimal interactions with a test set of analytes under the examined conditions. This led to the attainment of high recoveries of the analytes after suppression (93–99% recovery) and significantly reduced band broadening during suppression. The new suppressor has been shown to perform well under both isocratic and gradient elution conditions.

1. Introduction

Ion chromatography (IC) offers complementary separation selectivity to reversed-phase high performance liquid chromatography (RP-HPLC) for the identification of impurities in ionogenic pharmaceutical compounds. Therefore, use of both IC and RP-HPLC for analysis of the same sample should maximise the opportunity for separation and identification of impurities. Furthermore, hyphenation of IC to universal detectors (such as mass spectrometry or nebulising detectors) is highly beneficial for this purpose, but this is impeded by the presence of the high salt content present in typical ion-exchange eluents. One approach to facilitating the hyphenation of universal detectors with IC is to use an IC suppressor to de-salt the eluent prior to the detector [1].

Modern suppressors consist of a sandwich membrane arrangement in which the eluent passes between two planar ion-exchange membranes, with a regenerant solution passing on the outer sides of the two membranes. In anion-exchange IC (which for simplicity will be the only type of IC discussed here) the regenerant is either a solution of an appropriate acid or water which can be electrolysed to generate a source of hydronium ions. The former case is referred to as chemical suppression, and the latter as electrolytic suppression. Eluent cations migrate through the membranes into the regenerant stream, while hydronium ions migrate through the membranes into the eluent stream. When the eluent consists of a hydroxide salt (such as KOH) this process effectively converts the eluent into water. At the same time, analyte anions emerge from the suppressor in their acidic form. Strongly acidic analyte will therefore be fully dissociated, while weakly acidic analytes will be only partially dissociated.

Suppressors are designed to provide optimal performance for conductivity detection of inorganic ions and low molecular weight organic ions. The electrolytic anion self-regenerating suppressor (ASRS-300®) has been successful in such applications, providing good chromatographic efficiency due to the low dead volume [2], high suppression efficiencies and good recoveries for low molecular weight analytes. However, in the case of the pharmaceutically related analytes considered in the present study, there are potential losses of the protonated analytes in the suppressor due to precipitation and/or hydrophobic adsorption effects. For this reason, it is necessary that the eluent contain a suitable organic modifier to minimise these effects. Recently we have shown that even when organic modifiers are added to the eluent, satisfactory results can be obtained for the suppression (i.e. de-salting) of eluents used in gradient IC [3]. We have also examined the extent to which peak distortion and losses of hydrophobic and weakly ionised analytes

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doi:10.1016/j.chroma.2012.02.008

occur during electrolytic suppression [4]. It was demonstrated that peak distortion and losses were related to the suppression conditions used and also to the physico-chemical properties of the analytes, such as hydrophobicity and charge, and that these effects could be attributed to mechanisms of adsorption, precipitation and also permeation of the analytes through the suppressor membranes. We observed that in the case of electrolytic suppressors, analyte losses could be effectively eliminated only when the eluent contained very high levels of organic modifiers such as methanol or acetonitrile. However, long-term operation of suppressors using such eluents leads to deterioration of the suppressor membranes and the polymeric screens in the eluent and regenerant pathway.

One approach to overcome this difficulty is to redesign the suppressor so that analyte losses and peak tailing can be minimised when eluents contain much lower percentages of organic modifier (e.g. 40% compared to 60–80% applied previously [4]). Membrane-based suppressors have undergone a series of refinements since their introduction, but none of these refinements has been focused on improving suppressor performance for weakly acidic, hydrophobic analytes of the type typically encountered in the pharmaceutical industry [1,5]. In the present study, we examine prototypes of electrolytic suppressors which are designed to minimise hydrophobic interactions on both the eluent screens and the membranes and to also be more compatible with organic solvents. The organic modifier of choice was acetonitrile rather than methanol, since it showed better reduction of hydrophobic interactions between the analyte and the suppressor [4], low and stable suppressed conductivity baseline, and also advantageous selectivity on ion-exchange columns [3]. The different suppressor prototypes were first assessed for suppression performance, and then the recoveries of three analytes were compared, leading to the choice of an optimal suppressor design and operating parameters. Finally, an extended analyte test set was evaluated under two elution profiles and suppression conditions and used for comparison with a chemical suppressor.

2. Experimental

2.1. Instrumentation

The IC system used in this study was a Dionex ICS-3000 instrument (Thermo-Fisher Scientific, Sunnyvale, CA, USA), 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 [3]. Organic solvents used in the eluents were introduced at 0.4 mL/min to the 0.6 mL/min stream of eluent produced by the EluGen® cartridge [6] through a 3-port tee-piece connector (Upchurch Scientific, Oak Harbor, WA, USA), using an HPLC pump (Dionex Ultimate 3000 gradient pump), followed by a gradient mixer (Dionex GM-3 4 mm). An additional pump (Jasco PU-2089i, Easton, MD, USA; or a Dionex DXP-MS pump) was used to provide water at 1 mL/min to the continuously-regenerated anion trap column (CR-ATC) and degasser.

For chemical suppression, a Dionex anion micro-membrane 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 four different Dionex electrolytic suppressor designs (the commercially available ASRS-300® and three modified high-capacity anion suppressors). The electrolytic suppressors were operated at ambient temperature in the external water mode at a regenerant flow-rate of 3.0–5.0 mL/min, delivered through one of the DP pumps. A conductivity detector located in the DC was corrected to 35 °C with a temperature coefficient of 1.7%. Direct spectrophotometric detection was conducted using a Dionex UV–vis absorbance detector (VWD) located either before or after the suppressor. UV-absorbance spectra of the analytes were acquired by a Metertech UV/VIS SP 8001 spectrophotometer (Metertech Inc.; Taipei, Taiwan). The analytes were separated on either a Dionex IonPac® AS-11 HC (250 mm × 4 mm) or an AS-20 (250 mm × 4 mm) analytical column equipped with the matching guard column (50 mm × 4 mm) and heated to 30 °C. All experimental points were obtained at least in triplicate. A total of eight electrolytic suppressor units (of the commercially available or the three high-capacity designs) and two AMMS units were examined.

The data acquisition system was Chromeleon (Version 6.80). Physico-chemical properties of the analytes were obtained using ACDLabs software v.12.0 (Advanced Chemistry Development Inc., Toronto, Canada). According to the analytes molecular structure, the software predicted its log *P* (Partition coefficient), log *D* (Distribution coefficient) at pH 5.2, p*K*a and polar surface area (PSA, the sum of surface over all polar atoms in a molecule). Statistical analysis was performed on Excel™ (Microsoft Corporation, Redmond, Washington, USA), using the analysis ToolPak add-in.

2.2. Materials

Aqueous solutions were prepared with ultra-pure water purified on a Millipore (Bedford, MA, USA) Milli-Q system and having a specific resistance of 18.2 MΩ/cm. HPLC gradient UV grade acetonitrile was obtained from Ajax Finechem (Australia) or Honeywell Burdick & Jackson (Muskegon, MI, USA). Sulfuric acid regenerant (20–50 mM) was prepared from a 1 M stock solution made by dilution of 98% sulfuric acid (Ajax Finechem). The eluents were degassed by sonication under vacuum before use. Potassium hydroxide eluent was prepared electrolytically using a Dionex EluGen® eluent generator equipped with potassium hydroxide cartridge. Ibuprofen, mefenamic acid, naproxen, indoprofen, fenbufen, sulindac, diclofenac, flufenamic acid, tolfenamic acid, althiazide, chlorothiazide and furosemide were purchased from Sigma–Aldrich (Milwaukee, WI, USA). 1000 μg/mL stock solutions were prepared in 70% (v/v) acetonitrile, sonicated and further diluted with water to make standard solutions in 40% (v/v) acetonitrile.

2.3. Procedures

2.3.1. Suppressed conductivity of ionic gradient

The performance of the suppressors was evaluated by conductivity detection using a gradient of KOH in 40% (v/v) acetonitrile, as described previously [3]. The gradient test run started with a 3 min isocratic step of 5 mM KOH, followed by a ramp of 20 min up to 60 mM KOH, then 3 min isocratic step and return to start conditions in 2 min. 150 mA current was applied throughout the gradient since this current was required to suppress the maximum eluent concentration. The recorded parameters used for the evaluation were the total conductivity, noise and voltage, as measured at two time points at the end of each isocratic step (3 min, for 5 mM KOH; 25 min for 60 mM KOH).

2.3.2. Ion-exchange capacity of suppressors

Prior to the measurement of ion-exchange capacity, the suppressor was regenerated by 10 mL of 100 mM sulfuric acid and left to stand overnight. Then, any residual sulfuric acid was removed from the suppressor by flushing 3 mL water through both the eluent and the regenerant channels. Immediately after this, the static capacity of the suppressor was measured by applying 60 mM KOH in 40% (v/v) acetonitrile at 1.0 mL/min with the current turned off and the water regenerant flowing at 1.0 mL/min. The time between the beginning of the eluent flow and the sudden elevation

Table 1
Pharmaceutically related anionic test set for recovery assay. The analytes are listed in elution order. (a) Physico-chemical properties were calculated using ACD/Labs (unless indicated otherwise); (b) recovery rates after suppression of isocratic eluent by AMMS-300, ASRS-300 or HC-5mil-new; (c) recovery rates after suppression of gradient. %Error represents one standard deviation ($n = 3$). Suppression conditions are as described in text.

Analyte	(a) Selected properties and UV wavelength						(b) Isocratic elution			(c) Gradient elution
	MW (g/mol)	log *P*	log *D*, pH 5.2	p*K*a	PSA (Å^2)	UV λ (nm)	AMMS	ASRS	HC-5mil-new	HC-5mil-new
Ibuprofen	206.3	3.72	2.87	4.41[a]	37.3	254	88.3% ± 0.1%[c]	83.7% ± 0. 5%[c]	93.2% ± 0.3%	92.5% ± 0.1%
Indoprofen	303.0	2.77	1.90	4.39	60.4	294	99.2% ± 0.4%	94.4% ± 0.9%	100.0% ± 0.1%	100.1% ± 0.3%
Sulindac	354.0	3.59	2.57	4.22	54.4	260	97.9% ± 0.8%	95.0% ± 0.4%	98.4% ± 0.4%	98.3% ± 0.5%
Fenbufen	254.3	3.13	2.40	4.55	54.4	260	89.3% ± 0.8%	89.3% ± 0.7%	94.4% ± 0.6%	92.9% ± 0.3%
Naproxen	230.3	3.00	2.48	4.84	46.5	270	98.0% ± 0.2%[c]	95.0% ± 1.0%[c]	99.1% ± 0.1%	95.7% ± 1.2%
Diclofenac	296.2	4.06	3.00	4.18	49.3	246	98.9% ± 0.1%	96.2% ± 1.0%	100.0% ± 0.6%	98.6% ± 1.2%
Flufenamic acid	281.2	5.62	4.08	3.67	49.3	265	88.1% ± 0.3%	85.2% ± 1.1%	98.2% ± 0.3%	98.2% ± 1.3%
Mefenamic acid	241.3	5.33	3.85	4.20 [12]	49.3	337	95.7% ± 0.1%[c]	91.6% ± 0.3%[c]	98.8% ± 0.2%	97.1% ± 1.0%
Althiazide	383.9	1.11	1.11	8.33[b]	118	294	95.3% ± 0.3%	90.7% ± 0.8%	100.0% ± 0.5%	97.9% ± 1.1%

[a] Reference p*K*a values for ibuprofen are between 4.4 and 5.3 [13].
[b] pKa_2 is 9.55.
[c] Taken from previous work [4].

in baseline conductivity was recorded. Multiplying the suppressor exhaustion time by the applied ionic concentration gave the static capacity, in μequiv.

2.3.3. Analyte separation and recovery

The recovery value for an analyte after passage through the suppressor was measured for a 25 μL injection of a 100 μg/mL solution of the analyte by comparison of the UV peak areas measured before and after suppression, at an isosbestic wavelength (as detailed in Table 1). The isosbestic wavelength for each analyte was chosen using an offline spectrophotometric assay by selecting a wavelength in which the total absorbance of 100 μg/mL analyte in its acid form equalled that of its salt form in the examined matrix (40% (v/v) acetonitrile). For some analytes (tolfenamic acid, chlorothiazide and furosemide) the absorbance spectra did not provide a reliable isosbestic point, hence these analytes were omitted from the test set. Individual analyte recovery measurements were made under isocratic conditions using 20 mM KOH in 40% (v/v) acetonitrile for ibuprofen and naproxen on the AS-11 HC column and for indoprofen, fenbufen, sulindac, diclofenac and flumenamic acid on the AS-20 column, while 30 mM KOH in 40% (v/v) acetonitrile was used for mefenamic acid on the AS-11 HC column and althiazide on the AS-20 column. The recovery of analytes was also assessed for a gradient test run comprising 12 min of 5 mM KOH in 40% (v/v) acetonitrile, then a ramp of 10 min up to 60 mM KOH, followed by a 3 min isocratic step and returning to start conditions in 2 min. This gradient was suppressed by one of the new suppressor designs, at a constant current of 150 mA or 120 mA, using water regenerant at a flow rate of 3 or 5 mL/min.

3. Results

3.1. Suppressor designs

3.1.1. Modified screens

Micro-membrane suppressors consist of an eluent channel separated by ion-exchange membranes from two regenerant channels [2]. Both the regenerant and the eluent channels are defined by gasketed screens made of fibrous material. Electrolysis of water regenerant is conducted by two platinum electrodes placed on the exterior sides of the gaskets defining the regenerant chambers [7,8]. The flow of generated hydronium from the anode in the regenerant channel through the membrane into the eluent channel maintains continuous regeneration of the membrane to the hydronium form for the anion suppressor.

Current efficiency in the suppressor is defined as the extent to which the applied current generates only the stoichiometric number of hydronium ions required to neutralise the eluent and is governed by the transport of generated hydronium towards the cathode. The generated hydronium ions should ideally serve only to replace the eluent cations, thus, excess hydronium ions which are not exchanged with the analyte or eluent counter-ions during the suppression process translate into a wastage current and hence lower current efficiency, with implications such as generation of heat and elevated baseline noise [2,8]. The higher the ion-exchange capacity of the eluent screen, the lower is the current efficiency, and more current than that calculated by theory needs to be invested in order to suppress the ionic eluent. Improving the current efficiency has been a continuous goal in suppressor development, and successive optimisations have led to an achieved efficiency of over 90%, compared to 50% in the earlier models of the electrolytic suppressor [2]. In the commercially available electrolytic suppressors, only the regenerant channel screens have high ion-exchange capacity, in order to provide a reservoir of regenerant hydronium ions and facilitate transport of ions to and from the eluent channel.

Setting aside the objective of designing suppressors with high current efficiency, in the present study suppressor prototypes were constructed such that the screens in both channels were fully sulfonated, resulting in ion-exchange capacity of about 75% higher than the eluent screens in the commercially available ASRS-300. These suppressors were therefore designated as "high capacity (HC)". The aim of using a high capacity screen in the eluent channel was to decrease its hydrophobicity in order to reduce the hydrophobic interactions with the analytes observed in previous work [4], resulting in losses of hydrophobic analytes during suppression. In addition, especially in the presence of organic solvents in the eluent, the high capacity of the screens could also assist in reducing the potential drop across the membranes, resulting in less heat production, undesired side-reactions and diffusion effects [8]. In view of the contradicting design incentives above, an important part of the assessment of the suppressors in this work included optimisation of the current efficiency by controlling the suppression conditions.

3.1.2. Modified membranes

The membranes in the commercially available micro-membrane suppressors are constructed of PTFE, radiation grafted with styrene, and similarly to the screens, in the case of anion analysis they are further modified with sulfone groups to impart cation-exchange functionality [7]. Membranes having the same chemical composition as those used in the commercially available ASRS-300 were formed using two thickness levels, 0.003 inch (3 mil, 76 μm) and 0.005 inch (5 mil, 127 μm), and used in combination with the high capacity screen described above. The corresponding prototype suppressors were designated "HC-3mil-std" or "HC-5mil-std" to indicate both the thickness of the membrane and the fact that its composition was standard. An

additional type of membrane possessing approximately 47% higher ion-exchange capacity compared to the standard membrane was also tested. This proprietary new membrane was available only in 0.005 inch thickness and was used in conjunction with the high capacity screen, giving a prototype suppressor designated as "HC-5mil-new".

The motivation behind membrane modification was to increase the number of ion-exchange sites and to raise the charge density on the membrane. One outcome of the high capacity membrane design was reduction in the occurrence of hydrophobic pockets in the membrane available to adsorb interacting analytes. Although a thicker membrane potentially supplies more ion-exchange sites for transfer of hydronium ions through the membrane, some of these sites might not be accessible. Higher charge density on the membrane also enhances the Donnan barrier effect, repelling the eluent and the analyte anions from the membrane. This would minimise the penetration of the analyte through the membranes which has previously been identified as a minor source of recovery loss [4]. In addition, a thicker membrane would result in a lower rate of permeation of organic solvent through the membranes, thereby maintaining a higher solvent concentration in the eluent channel leading to reduced hydrophobic interactions of the analytes with the screen. Finally, the destruction of ion exchange sites induced by high current in the presence of organic solvent, would have a less significant impact on suppressor performance and longevity when the ion-exchange sites are in abundance.

3.1.3. Ion-exchange capacity

According to the elevated ion-exchange capacity of the screens and membranes in the prototype suppressors, the resulting overall ion exchange capacity is expected to be significantly higher than the standard suppressor. This effect can be observed in the measured static ion-exchange capacity, which is the number of equivalents of eluent ions that can be exchanged in the absence of regenerant ions. It serves as an indicator of the number of accessible ion-exchange sites, assuming full regeneration prior to the measurement. The measured ion-exchange capacities of the prototype suppressors were 435 μequiv. for HC-3mil-std, 490 μequiv. for HC-5mil-std and 625 μequiv. for HC-5mil-new. Surprisingly, an ASRS-300 showed slightly higher measured capacity (455 μequiv.) than HC-3mil-std, probably because the capacity of the latter suppressor was determined after a considerable exposure to harmful conditions during preliminary evaluation. For comparison, a heavily used ASRS-300 exhibited a very low ion-exchange capacity (160 μequiv.).

3.2. Ion-suppression performance

First, each new suppressor design was tested for the resulting conductivity baseline, noise and drift along a test eluent gradient. In eluent containing 40% (v/v) acetonitrile, all the high-capacity designs showed satisfactory results, with baseline conductivities of around 4 μS, which is lower than that for the ASRS-300 although still higher than AMMS-300 [3]. The examined suppressors showed similar drift of up to 0.3 μS, but reduced noise (~1 nS) compared to the ASRS-300. In fact, the baseline noise of the new suppressors was at least 10-fold lower than the ASRS-300. In a manner similar to that observed previously on the ASRS-300 [3], elevation of the regenerant flow-rate further improved the baseline conductivity by increasing the current efficiency through accelerating the clearance of unnecessary generated ionic species from the suppressor [2,8]. For example, on a HC-5mil-new suppressor, a 15–25% decrease in baseline conductivity was observed when the regenerant flow-rate increased from 3 mL/min to 5 mL/min, accompanied by a smaller drift (0.3 through 0.1–0 μS).

The advantageous suppression performance of the new designs reflected a lower potential drop than measured on the ASRS-300.

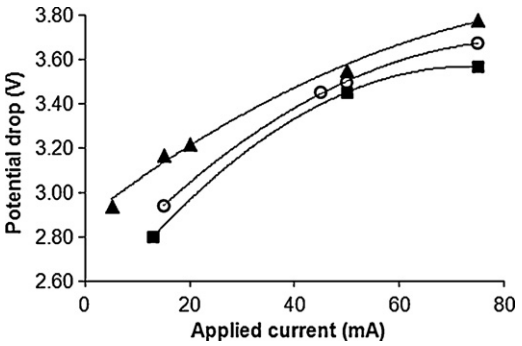

Fig. 1. Relationship between the electrolytic suppressor voltage and current measured for eluent containing 40% (v/v) acetonitrile, on ASRS-300 (filled triangles), HC-3mil-std (open circles) and HC-5mil-new (filled squares).

As illustrated in Fig. 1, the high-capacity screens lowered the voltage for a specified current by 0.1–0.3 V compared to an ASRS-300 operated under the same conditions. The thicker membrane suppressors (HC-5mil-std and HC-5mil-new) further decreased the voltage by 0.05–0.1 V. The zero-current point for a suppressor indicates the minimum voltage required to achieve minimum significant electrolysis of water and consequent generation of hydronium for suppression, and is normally above 1.5 V [9]. In the presence of eluents containing 40% acetonitrile the actual measured voltage for a given current will be higher due to the higher resistance of acetonitrile.

For a given suppressor current, the resistance characteristics at a particular point in the suppressor are defined by the form of the ion exchange material (i.e. the ratio between the hydronium and the eluent cation forms) and the degree to which the eluent has been suppressed at that point. Since these parameters vary, the current–voltage relationship is generally non-linear in all electrolytic membrane suppressors. In suppressors with a high-capacity eluent channel, the increased ion-exchange functionality provides a pathway for wastage current, particularly upon application of excess current, thereby increasing the rate of transport of hydronium ions compared to the eluent cation (potassium).

3.3. Effect of suppressor design on analyte peak shape

During passage through the suppressor, analyte bands may become broadened due to diffusional effects or as a result of adsorption effects occurring on hydrophobic regions of the suppressor. Band distortion effects were examined for three non-steroidal anti-inflammatory drugs (NSAIDs) using a range of suppressors with isocratic eluents. The results obtained for ibuprofen (Fig. 2) demonstrate that an improvement in peak shape occurred for the 5-mil high-capacity suppressors (Fig. 2(b)–(d)) compared to the HC-3mil-std suppressor (Fig. 2(e)) and the ASRS-300 (Fig. 2(f)). Moreover, the use of a higher regenerant flow-rate on the HC-5mil-new suppressor (Fig. 2(b)) can be seen to provide the best performance with regard to peak shape. These results can be interpreted in terms of the hydrophobicity of the suppressor, with the lowest hydrophobicity (reflected by the highest ion-exchange capacity) giving the best peak shape.

Fig. 3(a) shows the relationship between peak broadening and static ion-exchange capacity for the three NSAIDs tested (ibuprofen, naproxen, and mefenamic acid). Peak broadening, in most cases consisting of peak tailing, showed the expected inverse correlation to the static capacity of the high-capacity suppressors. For naproxen, the least hydrophobic of the three analytes, the band broadening compared to the ASRS-300 improved only slightly on the HC-5mil-new, which had considerably more ion-exchange sites than the other suppressors. On the other hand, the other two

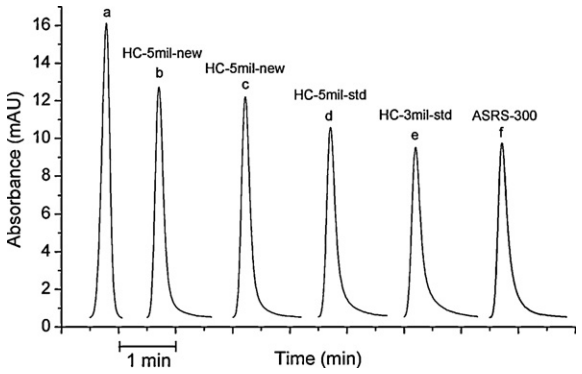

Fig. 2. Ibuprofen peak detected at 254 nm after separation on an AS-11HC column under isocratic conditions of 20 mM KOH in 40% ACN, before suppression (a) or after suppression with the indicated suppressor (b–f). An applied current of 50 mA and regenerant flow-rate of 3 mL/min was used for all suppressors except for (b), where a current of 40 mA and a regenerant flow-rate of 5 mL/min were used.

NSAIDs, which are more hydrophobic, showed considerably less brand broadening on the HC-5mil-new suppressor.

Recoveries of the three NSAID analytes were evaluated on the various suppressors. Regardless of the suppressor used, naproxen exhibited statistically constant recovery rates (96%), suggesting that the observed small losses were not the result of hydrophobic interactions with the suppressor. For ibuprofen and mefenamic acid, the improvement in peak shape observed in Fig. 3(a) was reflected in higher peak area recovery (see Fig. 3(b), which shows the relationship between recovery rates and static ion-exchange capacity of the suppressors. Note that this figure also includes data for a used ASRS-300 suppressor). This improvement in recovery can be attributed to a reduction in hydrophobic interactions between the analytes and the suppressor, as well as the fact that lower voltages were necessary on the high-capacity suppressors.

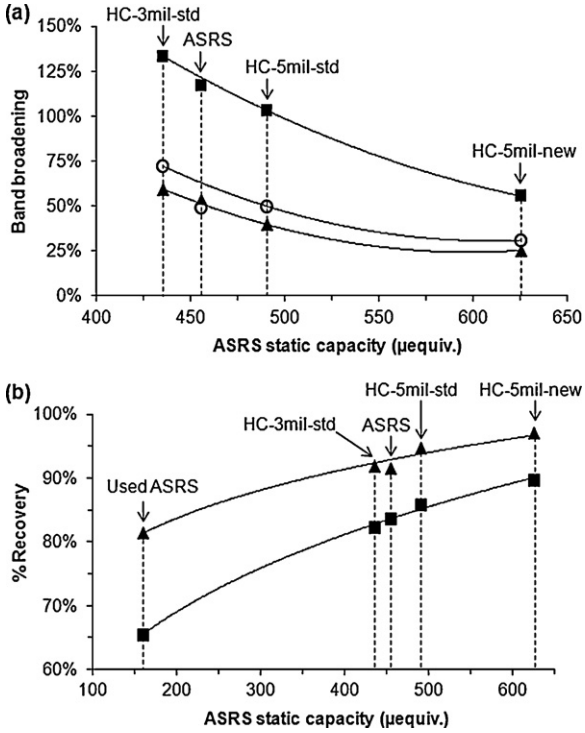

Fig. 3. Effect of the static ion-exchange capacity of the suppressor on (a) band broadening and (b) recovery for ibuprofen (filled squares), naproxen (open circles) and mefenamic acid (filled triangles). Peak broadening was calculated as the percentage increase in peak width (at 4.4% height) after suppression.

3.4. Effect of suppression conditions on recovery

In view of the superior performance exhibited by the HC-5mil-new suppressor, fine-tuning of the applied electric current and regenerant flow-rate was undertaken, assuming that the new suppressor may require slightly different operational conditions than the well-investigated commercially available ASRS-300. Previously we have shown the mild negative effect of higher-than-necessary electric current [4], and here we examine the potentially beneficial effects of lower current applied under the same eluent conditions. The high-capacity suppressors enable extended flexibility in the level of applied current under some gradient conditions, due to their higher reservoir of generated hydronium. Although the high-capacity of the screens potentially translates into a non-ideal lowering of current efficiency, under the eluent gradient conditions of the present experiment high capacity suppressors can be operated using a current which is lower than that calculated according to the default equation [10]. Operating under such conditions with 90% current efficiency, complete suppression of the ionic eluent was maintained overnight.

Fig. 4 shows recovery rates after suppression for the three NSAID analytes on a range of suppressors (including a micromembrane suppressor, MMS, operated in the chemical suppression mode) under a variety of regenerant flow-rates and applied currents. In general, it can be seen that lowering the applied current and increasing the regenerant flow-rate both improved the recoveries, particularly for the more hydrophobic analytes (ibuprofen and mefenamic acid, for which $P < 0.0002$ was obtained in t-test comparisons with the recoveries found under the default suppression conditions). The recommended water regenerant flow-rate is between 3 and 5 mL/min [10], and even higher when organic solvents are added to the eluent, in order to ensure hydration of the membranes [11]. In previous work on ASRS-300, variations within this range did not show any positive effect on the recovery rate of different hydrophobic analytes [4]. A disadvantage of increasing the regenerant flow-rate without accompanying it with lower current was the peak broadening observed for all analytes.

Finally, it can be noted that the recovery values obtained after optimisation of suppression conditions for the HC-5mil-new suppressor were similar or higher than those achieved on an ASRS-300 when 40% (v/v) acetonitrile was used as a regenerant instead of water. This step has been performed previously [4] as a means to prevent loss of analyte from the eluent chamber caused by an influx of organic solvent from the eluent into the regenerant channels. The HC-5mil-new is therefore an operational suppressor with good recovery rates without the need for organic solvent added to the regenerant and the resultant damaging side effects this causes.

3.5. Results of expanded test set on new suppressor design

In order to further validate the choice of the optimal suppressor design for use with pharmaceutically related analytes, an expanded analyte test set was established by adding a further six anionic compounds exhibiting a more diverse range of physico-chemical properties (see Table 1(a)). The performance of the HC-5mil-new suppressor was evaluated for these analytes under conditions where the applied current was minimised and higher regenerant flow-rates were used. Both isocratic and gradient eluent conditions were investigated.

3.5.1. Isocratic elution

Recoveries obtained for the test analytes under isocratic conditions are shown in Table 1(b), from which it can be seen that the recoveries of all analytes on the HC-5mil-new suppressor were higher than for the ASRS-300, and equal to or better than those for the chemical suppressor AMMS-300. For most analytes the

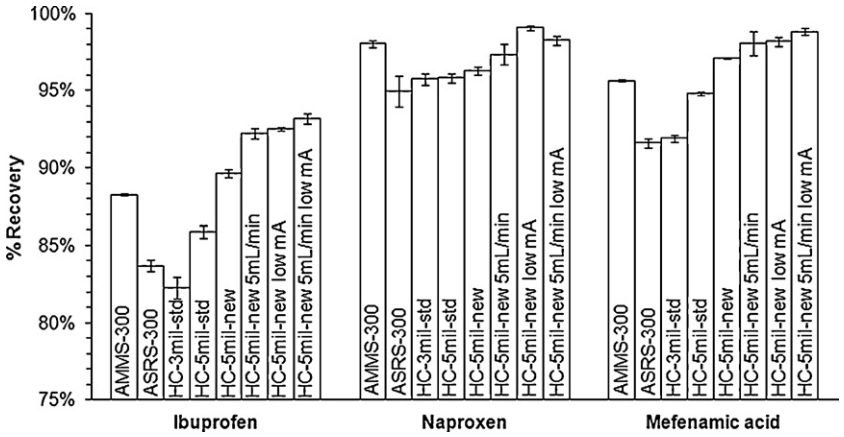

Fig. 4. Suppressed peak area recovery for ibuprofen, naproxen and mefenamic acid obtained with a range of suppressors and suppression conditions. Error bars represent one standard deviation ($n \geq 3$). The regenerant flow-rate was 3 mL/min unless indicated otherwise. The current applied on the ASRS was the recommended for suppression of the eluent concentration (75 mA for mefenamic acid and 50 mA for ibuprofen and naproxen), or 20% lower current, where indicated "low mA".

recovery improved from the ASRS-300 by an average of 5%, and for flufenamic (highest log *P* and log *D*) and althiazide (highest p*K*a) by about 10%. Optimisation of the suppression conditions, as conducted previously for the three NSAIDs, had a significant effect only on the recovery of sulindac and fenbufen.

For the test set as a whole, no clear relationships were found between any single physico-chemical property of the analytes and their recovery rates. This suggests that either multiple properties were involved or simply that at such high recovery rates no significant interactions took place between the analytes and the suppressor. An important advantage that the new suppressor design offers is decreased residence time and decreased interactions of the analyte in the suppressor. As illustrated in Fig. 5 for the test analytes, a reduction of up to half the peak broadening compared to the ASRS-300, and even further compared to the AMMS-300 was prevalent. This improvement is crucial since the separation efficiency of hydrophobic analytes is not very high on the existing ion-exchange columns. Suppression generally introduces further band broadening and it is not uncommon to see substantial tailing when the commercially available suppressors are used, leading to difficulties in achieving complete separation of analytes.

3.5.2. Gradient elution

The use of gradient elution was examined under conditions where the applied current was maintained throughout the gradient at the level necessary to suppress the highest eluent concentration achieved over the gradient. Under these conditions, an unnecessarily high current is applied for the very low eluent concentrations used at the start of the gradient. This practice can cause localised heating effects and rapid deterioration of the suppressor [4,8] and does not utilise the advantageous ability of high-capacity suppressors to perform stoichiometric suppression while applying lower current, owing to a higher reservoir of hydronium ions. The application of extremely high current had only a slight effect on the recovery rates of most analytes. Elevated regenerant flow-rate is important especially during the lower eluent concentration steps of the gradient, where ionic regenerant products are in extreme excess. As shown in Table 1(c), optimisation of the suppression conditions for gradient elution (5 mL/min regenerant flow rate or 120 mA applied current) resulted in recovery levels that were similar to those observed for isocratic runs.

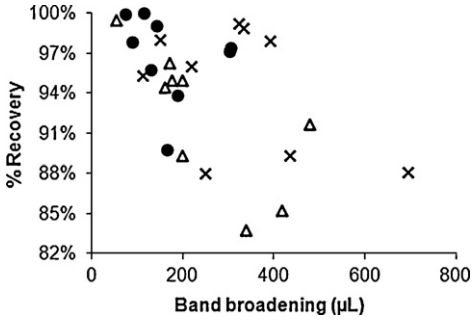

Fig. 5. Analyte recovery rates as a function of band broadening (µL added to peak width at 4.4% height) during suppression by AMMS-300 (×), ASRS-300 (open triangles) and HC-5mil-new (filled circles) under similar eluent and suppression conditions.

4. Conclusions

Of the three prototypes of new electrolytic suppressors examined in this study, a suppressor with a new type of thicker, high capacity membrane and high capacity screens showed the best results in terms of suppressed conductivity baseline, analyte peak shape and recovery. This performance was attributed to the high ion-exchange capacity of that suppressor model, originating from the characteristics of the membranes and the use of high-capacity screens. The average recovery rates of the pharmaceutically related test set of analytes was 97% on the HC-5mil-new suppressor, compared to about 92% on the ASRS-300 and 95% on the AMMS-300. These results, including the stability of the new suppressor under more challenging suppression conditions, validated the potential use of this suppressor design for the neutralisation of complex elution profiles prior to universal detection. The performance of this suppressor for IC coupled to mass spectrometry and to nebulising detectors such as evaporative light-scattering detection (ELSD) and Corona-charged aerosol detection (CCAD) will be reported in subsequent work.

Acknowledgements

ASRS, AMMS, IonPac and EluGen are registered trademarks of ThermoFisher Scientific. The Australian Research Council is acknowledged for the provision of a Federation Fellowship (FF0668673) to Paul Haddad. We thank Sheetal Bhardwaj for the assistance in the design and fabrication of suppressor prototypes.

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