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9 On-line electrodyalytic matrix isolation
10 for chromatographic determination of organic acids in wine
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Abstract

Chromatographic determination of organic acids is widely performed, but the matrix often calls for lengthy and elaborate sample preparation prior to actual analysis. Matrix components, e.g., proteins, non-ionics, lipids etc. are typically removed by a combination of centrifugation/filtration and solid phase extraction (SPE) that may include the use of ion-exchange media. Here we report the quantitative electrodialytic transfer of organic acids from complex samples to ultrapure water in seconds using cellulose membranes modified with N, N-dimethylaminoethyl methacrylate, which essentially eliminates the negative ζ -potential of a regenerated cellulose membrane surface. The transfer characteristics of the ion transfer device (ITD) were evaluated with linear carboxylic acids. While the ion transfer efficiencies may be affected by the acid dissociation constants, in most cases it is possible to achieve quantitative transfer under optimized device residence time (solution flow rate) and the applied voltage. In addition, the transfer efficiency was unaffected by the wide natural variation of pH represented in real samples. The approach was applied to organic acids in various samples, including red wine, considered to represent an especially difficult matrix. While quantitative transfer of the organic acids (as judged by agreement with standard pretreatment procedures involving SPE) was achieved, transfer of other matrix components was < 5%. The processed samples could then be chromatographically analyzed in a straightforward manner. We used ion exclusion chromatography with direct UV detection; in treated samples; there was a dramatic reduction of the large early peaks observed in compared to only 0.45 μ m membrane filtered samples.

Keywords: Ion transfer device; Sample pretreatment; Organic acids; Ion exclusion chromatography; Wine

1. Introduction

Organic acids play key roles in the taste and flavor of food and drinks [1]. The determination of organic acids is important for quality and process control in the fermentation industry, this includes the production of most alcoholic beverages [2,3]; organic acids play a major role in the microbiological and physicochemical stability and sensory properties. Monitoring of multiple organic acids simultaneously is often essential and is typically conducted with ion/liquid chromatography. Many samples contain, however, fine particles, proteins, lipids, other small organic compounds and macromolecules, etc. that drastically affect the usable lifetime of expensive chromatographic columns [4]. Organic compounds in sample matrices not only interfere with direct UV absorbance measurements [5], but also co-elute with an analyte acid of interest during chromatography. Improved detection by post column reaction with 4-nitrophenol [6] or bromothymol blue (BTB) [7,8] as indicator has been proposed. This shifts the detection wavelength to the visible region; however, the column fouling problems still remain and matrix isolation is still highly desirable.

Reported sample pretreatment methods for the liquid chromatographic determination of organic acids in food and beverages include centrifugation – filtration [9], solid phase extraction (SPE) [10-12], and on-line dialysis [13]. C18 SPE pretreatment was successfully applied to remove neutral organics from coffee [10]. A combination of C18 and strong base anion exchange (SAX) cartridges has also been used to separate polyphenolic compounds and organic acids [11,12]. Centrifugation, filtration and SPE treatments are applied batchwise to each sample. Online dialysis has been used in the flow injection mode [13]: a relatively large amount (~1 mL) of sample solution was injected and the dialysate in the acceptor stream was directly introduced into an HPLC system. Transfer efficiencies of the analyte acids in this case are driven only by a concentration gradient and proceed by diffusion; with realistic residence times in the dialyzer the efficiencies are poor, ranging from 4.6 to 9.5%. Furthermore, diffusion is not selective - nonionic organics are transferred as well. It is possible to operate in the stopped flow mode (acceptor solution is kept stationary while the sample flows continuously) to achieve concentration equilibrium where the analyte concentration in the acceptor approaches that in the sample [14]; this is a long procedure requiring large amounts of sample and results in substantial transfer of many unwanted moieties.

Recently, we reported an exhaustive ion transfer device (ITD) as a robust sample pretreatment method for ionic solutes [15] and demonstrated the electric field induced quantitative transfer of inorganic cations and anions from a sample to respective acceptors (consisting of pure water). Direct electrode contact of analytes was prevented by device design. This avoided redox conversion of susceptible analytes. We made the observation that it required a greater electric field to transfer anions relative to cations of comparable size and mobility.

As we attempted to use the ITD to carboxylic acid analytes, including those of significant chain length (and thence of relatively low mobility), typical of organic acids occurring in many fermented beverages, we found that it was not possible to accomplish near quantitative transfer at reasonable electric field strengths

where joule heating and/or attendant membrane degradation did not yet present a problem. The primary membranes bounding the sample channel in the device were made of regenerated cellulose. We reasoned that the barrier to anion transport arises from the intrinsic negative ζ -potential of such membranes. In the present paper we report the functionalization of a regenerated cellulose membrane with *N, N*-dimethylaminoethyl methacrylate (DMAEMA) to eliminate the negative ζ -potential and thence its successful utilization for achieving quantitative transfer of low mobility weak acid anions from real samples. We report also on studies of pH effects; the sample pH dictates what fraction of the analyte is ionized and is thence subject to electromigrative transport. pH effects are compared with corresponding effects in SPE methods. The ITD was coupled on-line to an ion exclusion chromatograph-direct UV detection (IEC-UV) system. This was applied to the several types of real samples, e.g., red and white wines, sake, and salad dressing. The results were compared with those obtained with conventional SPE-based pretreatment.

2. Experimental

2.1. Reagents and standard solutions

Carboxylate/carboxylic acid standards were prepared with analytical grade reagents (www.nacalai.co.jp or www.wako-chem.co.jp). Formate (C1), acetate (C2), propionate (C3), succinate (C4-2) and citrate were sodium salts. Malic, tartaric, and pyroglutamic acids were prepared from pure solid acids. Butyric (C4), valeric (C5), caproic (C6), lactic, levulinic, isobutyric, and isovaleric acids were prepared from pure liquid acids that were standardized by acid-base titration. All stock solutions were 10 mM in concentration. Working standards and mixtures were prepared daily. Milli-Q ultrapure water (Simplicity UV, www.millipore.com) was used throughout.

2.2. Ion transfer device

The structure of the ITD was the same as that previously described [15]. A schematic diagram of the ITD used is depicted in Fig. 1. Briefly, five flow-through stacked layers are separated from each other with membranes and sealed with Parafilm[®] gaskets supported by a 150 mesh nylon screen (thickness 0.11 mm, www.nippon-clever.co.jp). Effective channel dimensions were 5 mm W $\times$ 40 mm L $\times$ 0.13 mm thick. The overall structure can be represented as:

PPTM+ /isolator/ CEM / acceptor(+) / ITM / sample / ITM / acceptor(-) / AEM / isolator / PPTM-.

Pt-plated Ti mesh (PPTM) electrodes used for applying electric fields were ~50 mesh and 0.06 mm thick. The CEM and AEM were respectively cation exchange membrane (Selemion[®] CMV) and anion exchange membrane (Selemion[®] DSV) obtained from www.aged.co.jp. The details of the ion transfer membranes (ITM) are described below. Applied voltage (0-35 V DC) was supplied by a switching power supply (P4K-80M, www.matsusada.co.jp).

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127 **2.3. Functionalization of ion transfer membrane**

128 Regenerated cellulose membranes (RCMs) were used as the ITMs in both the cation and anion transfer sides
129 in the previous study [15]. As indicated in the introduction, the anionic hydroxyl groups in a RCM (molecular
130 weight cut off: 8000, Spectra/Pore Biotech dialysis membrane, www.spectrumlabs.com) was made weakly
131 cationic by reaction with *N, N*-dimethylaminoethyl methacrylate (DMAEMA, www.tcichemicals.com) as
132 shown in Fig. 2 [16,17].

133 The RCM was immersed in a large volume of continuously stirred deionized water for 48 h; the water
134 was refreshed every 12 h. The membrane was next washed with methanol and then acetone, followed by
135 vacuum drying for 24 h. The dried membrane was put into ferrous ammonium sulfate solution (0.2% w/v) for
136 30 min at 30 °C and rinsed with deionized water. The membrane was next put in a 40% w/v DMAEMA in 2
137 mM aqueous H₂O₂ for 5 h at 50 °C. The solution was degassed by purging with pure nitrogen and kept in a
138 sealed environment. After this period, the membrane was treated with 1% w/v oxalic acid solution to remove
139 iron, followed by copious washing with warm water. The washed membrane was serially put into 10 mM
140 HCl, 10 mM NaOH and washed with water for 2 h after each and then vacuum dried for 24 h. The infrared
141 spectrum of the functionalized membrane showed the characteristic >C=O absorption at 1800 cm⁻¹, not
142 present in the original membrane. The functionalized membrane was stored in a desiccator until device
143 assembly.

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145 **2.4. Ion transfer procedure**

146 The system set up is schematically shown in Fig. 1. Ultrapure water was continuously flowed into the ITD as
147 sample carrier, acceptor and isolator solutions. Sample carrier, acceptor (-) and isolator solutions were flowed
148 at 0.10, 0.10 and 0.40 mL min⁻¹, respectively, with an eight-channel peristaltic pump (Dynamax RP-1, Rainin,
149 France). The flow rate of sample carrier was carefully adjusted with a liquid mass flow meter (LF-F30M-A,
150 full scale 0.50 mL min⁻¹, www.horiba.com). The anode acceptor (+) solution, which receives the analytes of
151 interest was pumped by an independent pump (WPX1-P1/16S4, www.welco.net) so its flow rate could be
152 independently adjusted. The flow rate was monitored with a liquid mass flow meter and except as stated held
153 at 0.10 mL min⁻¹ by drive voltage adjustment of the pump. The water for this acceptor channel was purified
154 in-line with serial columns (9 mm ϕ , 50 mm long ea., P/N 5010-60121 and 5010-60000, www.gls.co.jp),
155 respectively containing strong acid form cation exchange and mixed bed ion exchange resins (Dowex
156 Monosphere 650C and Dowex Monosphere MR-450 UPW, www.sial.com).

157 Sample solutions were injected into the carrier stream via an injector (V-451, www.idex-hs.com)
158 equipped with a sample loop (0.5 mL, 1.5 mm id). The electrodialed acceptor was directly introduced into
159 the sample loop (0.020 mL) of an ion exclusion chromatography (IEC) system detailed in next section.
160 Injection in the IEC system was programmed to occur 4 min after the sample was injected into the carrier

stream. The transfer efficiency was calculated as the peak area ratio of standard solutions with and without the ITD.

2.5. Carboxylic acid determination with ion exclusion chromatography

Carboxylic acid analytes were analyzed by a standard approach involving separation in the ion exclusion mode followed by UV absorbance detection at 210 nm (IEC-UV) [18]. The chromatography system consisted of a dual plunger pump (PU-2080 Plus), UV-VIS detector (UV-2075 Plus, both from www.jasco.co.jp), and a column oven kept at 40 °C (U-620 VP#50, www.sugaicmi.com). The eluent (3 mM HClO₄) was pumped at a rate of 0.7 mL min⁻¹ through the separation column (RSpak KC-811, 8.0 mm i.d. × 300 mm, www.shodex.com). These separation conditions followed commercial vendor application notes [19]. For the 0.020 mL injection volume used, the limits of detection (LODs) were at the single digit µM level. The upper limit of the linear dynamic range ranged from 2 to 50 mM.

2.6. Real samples and analytical procedure

Samples were commercially purchased. Red wines tested were medium, light, and full bodied and all locally made. One light and one full bodied wine was further used for recovery experiments. The white wine was from Germany. The sake tested was made from rice without distillation. The salad dressing was soya sauce taste. The dressing was shaken well before sampling. These samples were aspirated from the bottle with needle equipped syringes and to avoid oxidation kept in capped syringes at 4 °C in the dark until dilution and further processing.

2.7. Comparative solid phase extraction method

A widely used approach, promulgated by a commercial vendor as an application note [20] was used as the benchmark. The method utilizes a reverse phase SPE cartridge (containing 230 mg styrene-divinylbenzene copolymer, Intersep® mini RP-1, www.glscience.co.jp) that was conditioned by sequentially passing 5 mL each of methanol, deionized water, and 0.02 M HCl. An appropriately diluted sample was then introduced into the cartridge through a syringe filter (RCM, 0.45 µm pore, Minisart® RC 25, www.sartorius.com). After discarding the initial 2 mL, the effluent was collected and analyzed by the IEC-UV system.

3. Results and discussion

3.1. Membrane functionalization for ion transfer device

Standard commercial RCMs has a negative ζ-potential at pH levels > 2.2 [21]. This makes rapid quantitative transfer of low mobility ions difficult at reasonable electric field strengths. The simplest way to eliminate a negative ζ-potential is to add positively charged functional groups. Functionalization of cotton fibers with

DMAEMA to form weak anion exchangers has been reported for removing anionic dyes from waste water [17]. Presently DMAEMA functionalization was carried out to improve the anion transfer efficiency of RCMs. The ζ -potential of functionalized (DMAEMA/RCM) and bare RCM as determined experimentally (Zetasizer nano ZS with surface zeta potential accessory, both from www.malvern.com), were -0.5 ± 0.8 and -6.13 ± 1.0 mV at pH 9, respectively. The improvement of ion transfer efficiencies was detailed below.

When we previously considered the quantitative transfer of high mobility inorganic cations and anions, the minimum applied voltage required was very different for cations and anions. Consider that K^+ and NO_3^- has equivalent conductances, proportional to their electrophoretic mobilities of 73.5 and 71.4 S cm² equiv⁻¹ [22]. Figure 3a shows the extent of transfer of K^+ and NO_3^- from a 100 μ M KNO_3 sample: ~ 8 and ~ 15 V are respectively required for the quantitative transfer of these ions of comparable mobility, clearly indicating the obstructive role the negative ζ -potential of the ITM plays towards anion transfer. Note that our previous study also indicated that an ion exchange membrane of any significant exchange capacity cannot be used as an ITM; the ions of interest load onto the ion exchange sites and are not easily eluted [15]. We wanted to make the effective ζ -potential of the RCM for an ITM close to zero. Preliminary experiments were conducted to determine how exchange capacities change as a function of the reaction time with DMAEMA and how this affects the ion transfer process. Based on these results, a 5 h reaction time was chosen. The anion exchange capacity of the DMAEMA/RCM under these conditions was 220 peq channel⁻¹, as determined by acid-base titration. In comparison, the ion exchange capacity of the commercial AEM used in the device was 50 μ eq channel⁻¹.

With the DMAEMA/RCM used as the ITM, any difference in voltage dependence on transfer characteristics of K^+ and NO_3^- was essentially eliminated (Fig. 3a). Applied voltages required for quantitative transfer of weak acids, acetate and propionate, also decreased from >25 V to ~ 20 V for the RCM to the DMAEMA/RCM (Fig. 3b).

3.2. Effects of carbon chain length on the efficiency of electrodialytic transfer

Analyte binding on a membrane may be affected by both electrostatic and hydrophobic forces. For a series of linear carbon chain carboxylates, while the electrophoretic mobilities do decrease with the carbon number (n), the rate of change decreases greatly with increasing n : the equivalent conductance values of C1-C4 carboxylates are 54.6, 40.9, 35.8 and 32.6 S cm² equiv⁻¹ [22], respectively and there is a concomitant rise in the hydrophobic binding of the anion. We studied C1-C6 straight chain carboxylic acids; the poor solubility of n -heptanoate onwards presents a problem to extend the experiments to higher chain lengths. We also studied succinate (C4-2, equivalent conductance 58.8 S cm² equiv⁻¹ [22]), an intermediate carbon chain length dicarboxylate, widely regarded as a model important contributor to *umami* in taste and flavor studies [23].

The transfer efficiency is taken to be the ratio of the concentration in the acceptor effluent to that in the injected sample solution; the results are shown in Fig. 4. All the tested carboxylates were quantitatively

transferred from the sample to the acceptor solution at 30 V. At lower voltages, shorter chain carboxylates were transferred at higher efficiencies relative to the longer chain analytes. The applied voltages required for quantitative ion transfer were in the order $C1 < C4-2 < C2 < C3 \sim C6$. The corresponding pK_a values are 3.77, 4.2, 4.76 and $4.87 \sim 4.95$ for C1, C4-2, C2 and C3~C6, respectively. Fu et al. have shown that electrophoretic mobilities can be predicted from molecular volume and pK_a values [24]; indeed, the applied voltage necessary for quantitative transfer was related to the pK_a values.

Solution flow rates are also important in governing the extent of ion transfer; in a given device, the available time for ion transport to occur is inversely proportional to the central chamber flow rate. We examined sample and the acceptor (+) solution flowing at same flow rates; the shorter carbon chain carboxylates were quantitatively transferred up to flow rates of 0.20 mL min^{-1} , corresponding to an average device residence time of only 5.4 s.

To accommodate a broad range of analytes we hence chose an applied voltage of 30 V, ultrapure water as an acceptor, and a solution flow rate of 0.10 mL min^{-1} for further experiments.

3.3. Sample pH effects on the present method and on solid phase extraction

Although it may at first seem that electrophoretic transfer can only occur with ionized species and thus the transfer efficiencies of the weak acid analytes of interest must be significantly affected by the pH, especially as it decreases below the analyte pK_a value, this was found not to be the case. The effects of sample pH on the transfer efficiencies of linear carbon chain carboxylates were examined from sample pH of 1 to 8. At pH values > 2.5 , there was no effect of pH on the transfer efficiency. This is easily rationalized: removal of the ionized portion of a sample results in further ionization of the unionized portion for equilibrium to be maintained. Formic and acetic acids showed dissociation half-lives of 13 and $2.4 \mu\text{s}$, respectively [25], compared to the 11 s residence time. While pH does not directly affect the dissociation rate, the lower transfer efficiency at $\text{pH} < 2.5$ is caused, at least in part, by the preferential transport of high mobility anions that were used to adjust the pH. In the previous study [15], quantitative inorganic ion transfer was achieved up to $\sim 20 \text{ meq L}^{-1}$; this corresponds to a sample pH of 1.7 for strong acid anions.

The sample pH effects for the other carboxylates of interest in fermented beverages and foods were also examined. Comparative results of the present device and the benchmark SPE procedure detailed in experimental section are shown in Fig. 5. A reverse phase type SPE cartridge can adsorb unionized carboxylic acids. Accordingly, in many cases, there was a pronounced pH effect on the SPE method, if the $100 \mu\text{M}$ mixed standard pH (unadjusted value 3.69) was not adjusted to a higher pH value (with NaOH solution). The SPE effluent was also analyzed by IEC-UV as with the ITD. For the ITD, the recoveries were not affected by the sample solution pH. But for the SPE procedure, the recoveries were affected even at pH 3.69 for several analytes, butyric, levulinic, isobutyric, and isovaleric acids, as can be seen in Fig. 5. In the ITD, the recovery for butyric acid was quantitative in contrast; the recovery of the other three, levulinic, isobutyric, and

isovaleric acids, while less than quantitative, was still respectable at ~ 75%. Nevertheless, even for these analytes, the variability of the recovery with the sample pH was very low, ~3%. The pH-independent transfer efficiencies made it possible to calibrate the ITD method without having to adjust sample pH even for the pH of the mixed standards (3.69). We determined that the residual portion of the analyte acids that were not fully transferred, e.g., levulinic, do not undergo electrochemical degradation but remain in the carrier stream. The reason for this behavior for these compounds cannot be explained based on electrophoretic mobilities; we remain perplexed.

The ITD and benchmark SPE approaches differ in that equilibrium affects the two procedures in an opposite manner. When the sample pH is such that the analyte acid is not fully ionized, in the ITD, transfer of the anion to the acceptor causes more of the unionized acid to ionize to maintain equilibrium. In the SPE case, sorption of the unionized form by the SPE phase cause more of the ionized form to protonate and become unionized to maintain equilibrium. Sample pH adjustment is a must for SPE sample pretreatment to obtain high recoveries for some analytes. However, pH adjustment in some real samples caused sedimentation, adding to the need for a further step of filtration/centrifugation.

3.4. Application to real samples

All samples were diluted 10 times to keep the concentration within the linear dynamic range of the IEC-UV system. The 0.50 mL sample loop was loaded with the diluted sample through a 0.45 μ m pore syringe filter, without any other treatment. As in Fig. 2, 0.020 mL of the electrodialed acceptor was injected into the IEC-UV system. Standards were analyzed in the same way to prepare calibration curves. Similar determination of samples and standards was conducted with the benchmark SPE protocol detailed in Section 2.7.

The reproducibility for the ITD was initially studied with a red wine sample. The relative standard deviations for 9 different analytes in this sample, were in the range of $6.5 \pm 3.9\%$ ($n = 10$). The relative standard deviations and concentrations for all analytes are in Table S1 and Figure S1 in supplemental data. The responses did not significantly differ between runs. The effect of the electrodialectic sample pretreatment step compared to only filtration applied with RCM syringe filter was dramatic for red wine: the huge early eluting peak common to these samples was drastically reduced by the proposed method. The concentration of tannins (the dominant polyphenols in red wines) in the original and the electrodialed sample were measured by the ferrous tartrate method [26]. The tannin levels in the electrodialed sample was <5% of that in the original sample; Fig. 6 inset also shows that the optical absorbance at 480 nm of the electrodialed sample decreased by a proportionate amount. These data demonstrate that electrodialectic sample treatment effectively separates carboxylates from nonionic compounds and thus also avoids potential interferences in IEC – direct deep UV absorbance detection.

Recovery tests were performed with red and white wines, and sake. The standard solution was spiked at three different levels, 0.5, 1.0, and 1.5 mM. The original concentrations and mean recovery values and standard deviations were shown in Table 1. Furthermore, the recoveries at each added concentration were summarized in Table S2-S5 in the supplementary data. There were no significant differences in recovery among the different added concentrations. The recovery values were found to be in the range of 80 to 109% for all analytes.

The comparison results obtained with proposed ITD and SPE methods are shown in Fig. 7. Results obtained with red and white wines, Japanese sake, and salad dressing are all plotted together. Butyric and levulinic acids could be determined only with the ITD because of the sample pH effects on SPE. The pH of the samples studied ranged from 3.24 to 4.21. Except for butyric, levulinic and isobutyric acids, which could not be determined accurately by the benchmark SPE method, the results agreed reasonably. The regression equation for the data, which were not affected by sample pH on SPE, shown is :

$$[\text{ITD, mM}] = (1.022 \pm 0.041) \times [\text{SPE, mM}] \quad R^2 = 0.969 \quad \dots(1)$$

suggesting that the ITD likely exhibits less analyte loss compared to SPE.

4. Conclusions

Electrodialytic ion transfer provides a robust means of sample preparation for ionic analysis; this now includes the analysis of organic acids. We have shown applications of an online ion transfer device bearing a chemically modified regenerated cellulose membrane that makes possible reproducible, pH-independent and in most cases quantitative, organic acid transfer from complex samples. While it provides results comparable to or better than those from SPE pretreatment, it is easily incorporated in-line and one set of membranes have thus far lasted >150 sample injections.

Glossary

AEM	anion exchange membrane
CEM	cation exchange membrane
C1	Formate
C2	Acetate
C3	Propionate
C4	Butylate
C4-2	Succinate
C5	Valate
C6	Caproate
DM	dialysis membrane
DMAEMA	<i>N, N</i> -dimethylaminoethyl methacrylate

335 DMAEMA/RCM DMAEMA functionalized regenerated cellulose membrane
 336 FDM functionalized dialysis membrane
 337 IEC-UV ion exclusion chromatography with UV absorbance detection
 338 ITD ion transfer device
 339 ITM ion transfer membrane
 340 PPTM Pt-plated Ti mesh electrode
 341 RCM regenerated cellulose membrane

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Figure captions

Fig. 1. Schematic flow diagram of sample pretreatment method by means of ion transfer device. Abbreviations of CEM, DMAEMA/RCM, RCM, AEM, and PPTM represent cation exchange membrane, DMAEMA functionalized regenerated cellulose membrane, regenerated cellulose membrane, anion exchange membrane and Pt-plated Ti mesh electrode, respectively..

Fig. 2. Scheme for the RCM functionalization with DMAEMA.

Fig. 3. Effects of membrane functionalization on the transfer efficiencies of (a) inorganic ions and (b) carboxylates. 100 μM analyte concentration. Dashed and solid lines indicate RCM and functionalized RCM (DMAEMA/RCM), respectively. All solutions were flowed at 0.30 and 0.10 mL min^{-1} for (a) and (b), respectively.

Fig. 4. Transfer efficiency dependence on carboxylic acid carbon number.

Fig. 5. Transfer efficiencies of the present and the SPE method on the different pH values. Sample solution was mixture of analytes (100 μM ea.). Solution pH was unadjusted (pH 3.69) and adjusted with NaOH for pH 6.17 and 10.47.

Fig. 6. Chromatogram and absorbance spectrum for red wine sample treated with only filtration and present method (ITD).

Fig. 7. Regression plots with the results obtained with present method and SPE. Three red wines, a white wine, one sake, and a salad dressing sample were analyzed. The left panel shows only the results obtained with the ITD because no response was observed with SPE. See details in the text.

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Fig. 1. Schematic flow diagram of sample pretreatment method by means of ion transfer device. Abbreviations of CEM, DMAEMA/RCM, RCM, AEM, and PPTM represent cation exchange membrane, DMAEMA functionalized regenerated cellulose membrane, regenerated cellulose membrane, anion exchange membrane and Pt-plated Ti mesh electrode, respectively.

Fig. 2. Scheme for the RCM functionalization with DMAEMA.

Fig. 3. Effects of membrane functionalization on the transfer efficiencies of (a) inorganic ions and (b) carboxylates. 100 μ M analyte concentration. Dashed and solid lines indicate RCM and functionalized RCM (DMAEMA/RCM), respectively. All solutions were flowed at 300 and 100 μ L min^{-1} for (a) and (b), respectively.

Fig. 4. Carbon number effects on the transfer efficiencies. Flow rates for all solutions: $200 \mu\text{L min}^{-1}$.

Fig. 5. Transfer efficiencies of the present and the SPE method at different pH values.

Fig. 6. Chromatogram and absorbance spectrum for red wine sample treated with filtration only, SPE and present method (ITD).

Fig. 7. Regression plots with the results obtained with present method and SPE. Three red wines, a white wine, one sake, and a salad dressing sample were analyzed. The left panel shows only the results obtained with the ITD because no response was observed with SPE. See text for details.

Table 1 Recovery test

Acids	Red wine 1		Red wine 2		White wine		Japanese sake	
	Original conc., mM	Recovery, %	Original conc., mM	Recovery, %	Original conc., mM	Recovery, %	Original conc., mM	Recovery, %
Citric	ND*	99.0 ± 5.4	ND	98.0 ± 3.2	8.22 ± 0.20	102 ± 4.6	ND	88.5 ± 3.5
Tartaric	12.6 ± 0.71	106.6 ± 6.7	5.49 ± 0.56	100.9 ± 4.5	7.03 ± 0.21	102 ± 4.8	ND	92.3 ± 5.2
Malic	4.34 ± 0.20	95.5 ± 5.2	12.1 ± 1.3	89.7 ± 6.5	5.86 ± 0.12	106 ± 3.3	0.802 ± 0.005	100 ± 3.3
Succinic	6.05 ± 0.04	105.9 ± 5.5	5.17 ± 0.42	101.9 ± 6.7	4.63 ± 0.11	104 ± 2.5	2.78 ± 0.05	101 ± 1.3
Lactic	1.90 ± 0.12	98.7 ± 2.2	5.73 ± 0.35	99.6 ± 6.1	5.22 ± 0.18	105 ± 2.1	5.13 ± 0.08	105 ± 1.5
Acetic	6.95 ± 0.19	103.4 ± 0.7	7.39 ± 0.63	95.7 ± 5.1	6.12 ± 0.06	108 ± 2.6	2.98 ± 0.05	106 ± 5.4
Levulinic	ND	102.7 ± 7.6	ND	96.5 ± 5.4	ND	104 ± 3.7	3.66 ± 0.12	90.5 ± 2.9
Pyroglutamic	0.463 ± 0.015	99.2 ± 4.8	0.346 ± 0.027	95.7 ± 4.3	0.208 ± 0.001	104 ± 3.8	0.305 ± 0.022	109 ± 4.7
Isobutyric	3.09 ± 0.22	94.8 ± 3.6	2.89 ± 0.17	82.1 ± 5.4	2.19 ± 0.32	88.4 ± 2.0	3.50 ± 0.17	87.7 ± 1.3
Butyric	5.27 ± 0.12	95.7 ± 1.5	ND	85.8 ± 2.3	2.95 ± 0.29	80.1 ± 8.7	ND	85.2 ± 3.1
Isopentanoic	ND	92.7 ± 9.0	ND	86.9 ± 3.7	ND	90.8 ± 3.6	ND	84.1 ± 2.9

*ND: not detected