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On-line electrodialytic matrix isolation 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 compared to only 0.45 μm membrane filtered samples.

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

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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 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 transfer 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 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 × 40 mm L × 0.13 mm thick.

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)

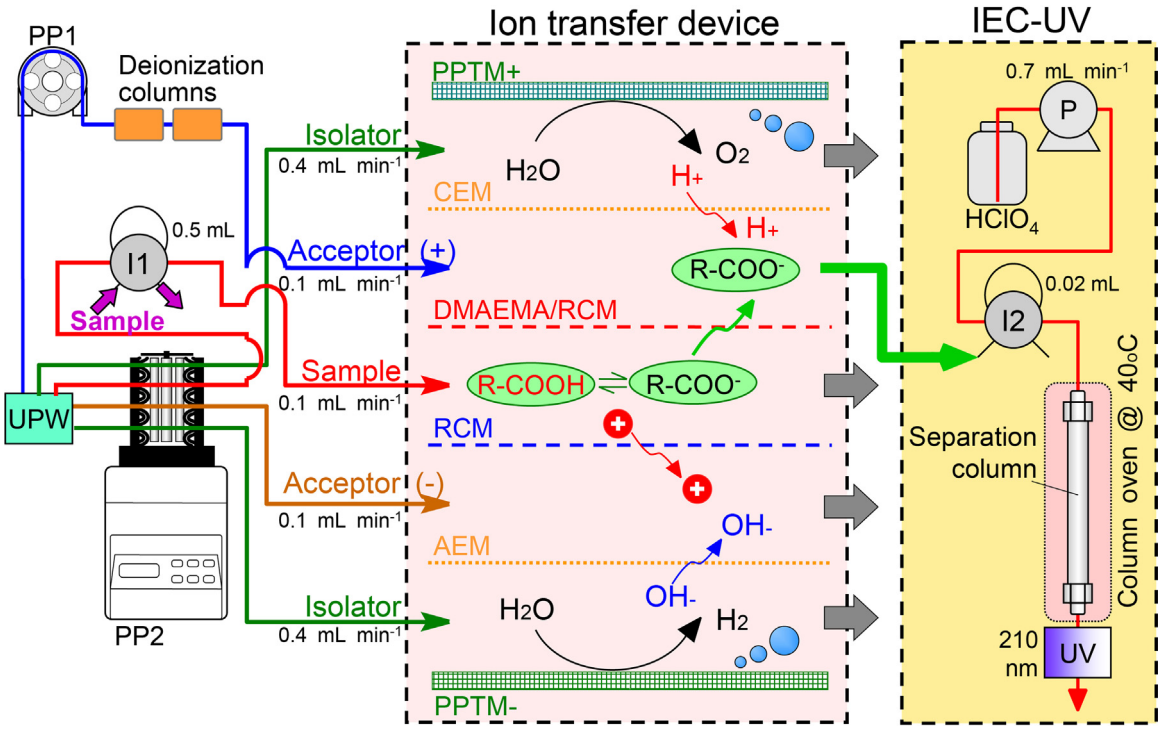

Fig. 1. Schematic flow diagram of sample pretreatment method by means of ion transfer device. Abbreviations of CEM, DMAEMA/RCM, RCM, AEM, and PPTM represent cation exchange membrane, DMAEMA functionalized regenerated cellulose membrane, regenerated cellulose membrane, anion exchange membrane and Pt-plated Ti mesh electrode, respectively. The overall structure can be represented as PPTM+/isolator/CEM/acceptor(+)/ITM/sample/ITM/acceptor(−)/AEM/isolator/PPTM-.