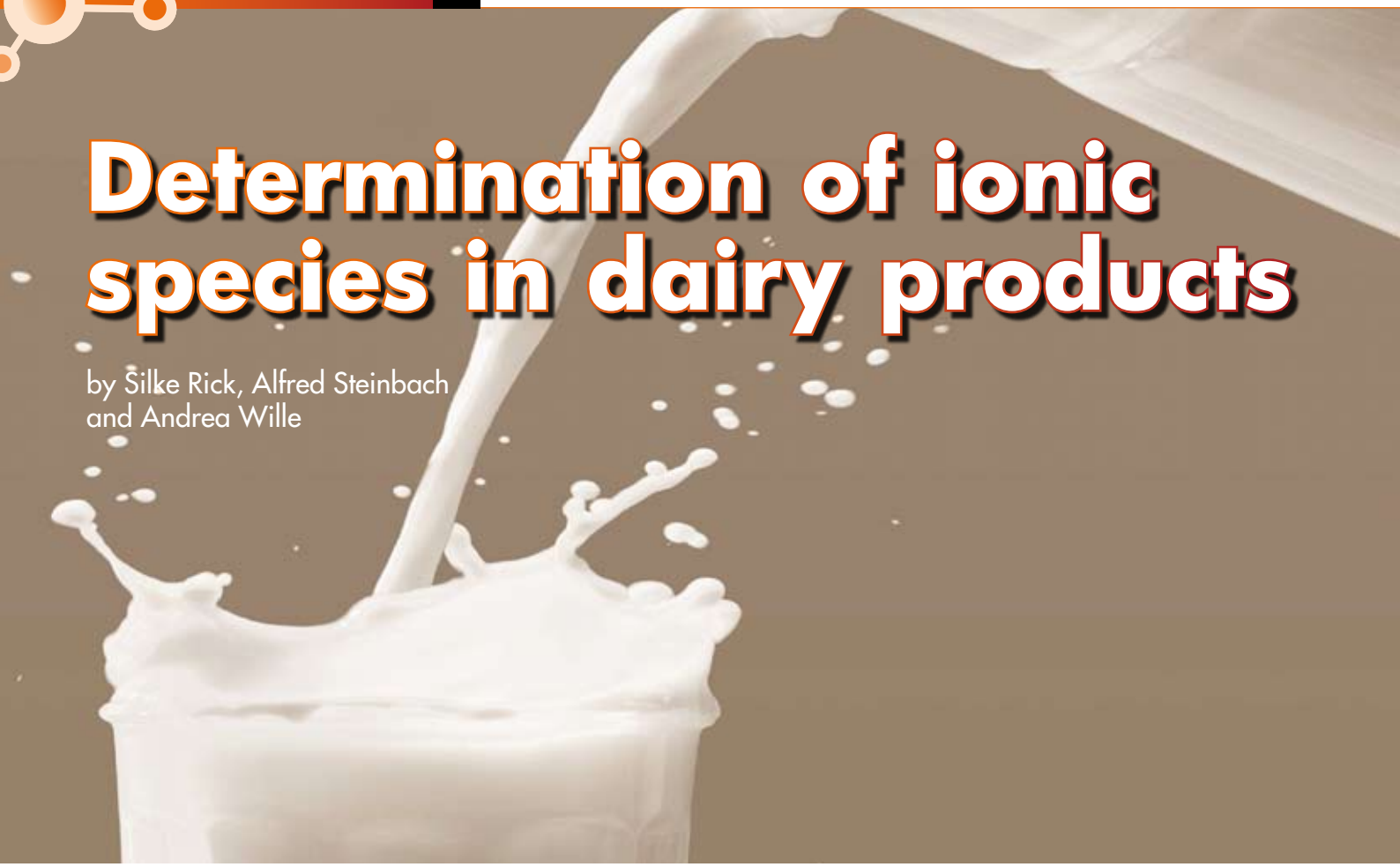

Determination of ionic species in dairy products

by Silke Rick, Alfred Steinbach and Andrea Wille

If a difficult food sample such as dairy products is run on an ion chromatograph (IC), there is a big chance that the column will be irretrievably wrecked. Of course, to avoid this a lot of time can be spent on tedious sample preparation steps to eliminate undesirable matrix components. Another option is to go for an automated compact stopped-flow dialysis system that provides optimum separation while protecting the column from detrimental compounds.

As an analytical technique ion chromatography (IC) has experienced an impressive surge in popularity due to the simplicity and robustness of the method, improved reliability and the wide range of available columns, detectors and applications. For a sample in a homogeneous ionic form, very little sample preparation is required and results can be obtained within a matter of minutes. However, in complex matrices carrying high organic loads such as dairy products, a more extensive sample preparation is mandatory to prevent destruction of the column.

Although numerous sample preparation techniques have been developed, such as the Carrez precipitation for protein-containing samples, most of them are tedious and error-prone. To overcome these shortcomings, Metrohm launched the first coupling of IC with dialysis in 1997. Since then the procedure has been further improved and allows an efficient in-line elimination of undesired matrix components in a variety of demanding sample types.

Using as examples an ultra-high temperature (UHT) processed milk and a baby milk powder sample, this article presents a fully automated sample preparation setup coupled to the new ion chromatograph 881 Compact IC pro [Figure 1]. Calibration parameters, carryover and recovery rates were tested with multi-anion standards. In this work the instrument set-up was based on an Ion Chromatography system (the 880 Compact IC pro from Metrohm), together with a Metrohm 858 Professional IC sample processor, a Metrohm 800 Dosino and spiral flow dialysis cell and accessories.

Figure 1. The experimental setup consists of an 881 Compact IC pro with the 858 Professional IC Sample Processor with dialysis cell and 800 Dosino. Instrument control, data acquisition and processing were carried out by MagIC Net software.

All standard solutions and eluents were prepared with deionized water having a specific resistance higher than 18 MΩ·cm. Two standard solutions covering the concentration ranges 1.0 to 3.6 mg/L and 10 to 36 mg/L were used to determine the system characteristics.

The ultra-high temperature (UHT) processed milk and the baby milk powder were purchased from Migros, Switzerland.

Compact stopped-flow dialysis

Dialysis is based on the selective diffusion of molecules or ions from one liquid (donor or sample solution) to another (acceptor solution) through a membrane, with the driving force for the transfer being the concentration gradient across the membrane. Contrary to dynamic dialysis, where two solutions continuously pass through the dialysis module, in stopped-flow dialysis at least one solution is temporarily stopped until the concentration in the acceptor solution is the same as that in the donor solution. This stopped-flow procedure takes between 10 and 14 minutes and can be directly coupled to an IC setup. As the dialysis is performed during the recording of the previous sample's chromatogram, the overall analysis time is not prolonged.

Whereas in a conventional setup two two-channel peristaltic pumps transport the sample and the acceptor solution to and from the dialysis cell, in compact dialysis a Dosino unit doses ultrapure water through the acceptor compartment of the cell. The stopped-flow status is achieved by stopping the Dosino and blocking the outlet capillary of the cell by feeding it through the valve of the Sample Processor. The latter, depending on its valve position, allows or blocks the acceptor solution flow [Figure 2].

System characteristics

Calibration

Five concentration levels (0.5, 1, 5, 10 and 20 mg/L) prepared from a multi-ion standard were used for external calibration [Table 1].

Carryover

This was evaluated by injection of a blank (ultrapure water) immediately after injection of a standard [Table 2].

Recovery rates

In order to determine recovery rates, results obtained by direct injection were compared to those obtained by injection of the dialysate (Table 3).

Dairy samples

UHT processed milk

Before analysis, the UHT processed milk sample was diluted 1:100 with ultrapure water and placed in the sample vials on the rack of the sample processor. The subsequent dialysis of the milk sample and the injection of the dialysate onto the separation column was fully automated. The calculation was carried out automatically using integration software MagIC Net 1.1 against previously prepared calibration plots.
Under the conditions described in the caption of Figure 3, excellent baseline separation of chloride, phosphate and sulphate is achieved within 12 min. Repetitive analyses showed no trending in peak areas or retention times, which suggests that sample proteins did not pass through the membrane.

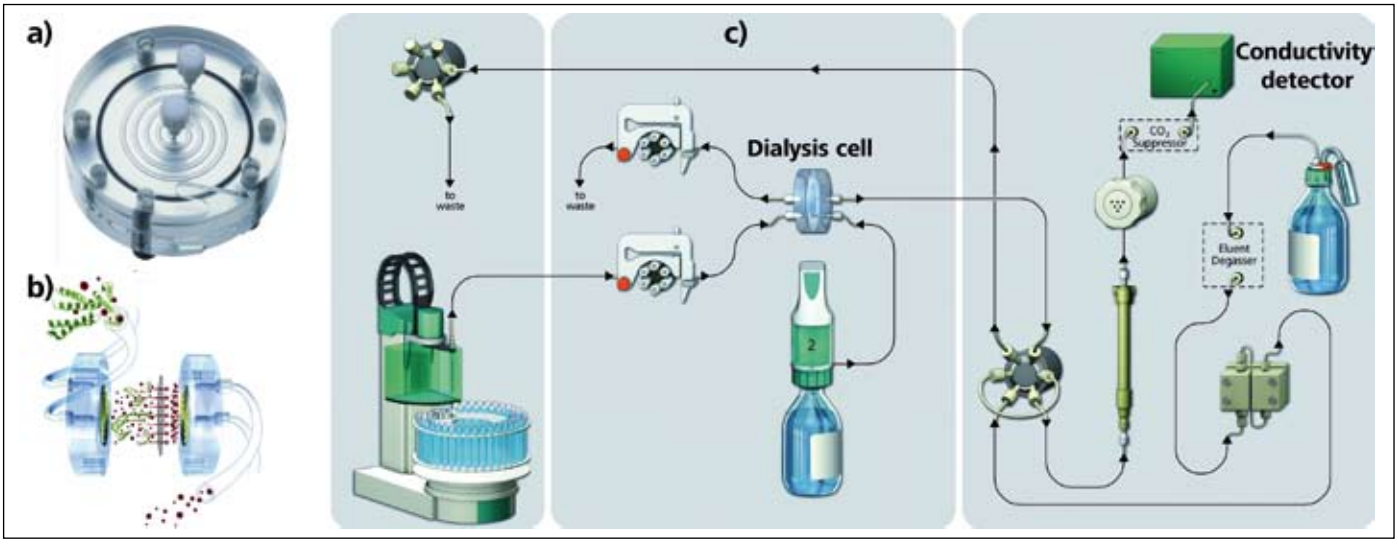

Figure 2: The dialysis cell and experimental setup. Figures (a) and (b) in the left part of the image show the patented spiral-flow dialysis cell. The schematic diagram (c) shows its link-up to the compact IC.

Baby food milk powder

Following the manufacturer`s instructions, the baby food milk powder was reconstituted with water. Before analysis, the prepared milk sample was diluted 1:100.

As with the UHT milk sample, here too, the applied chromatographic conditions enabled an excellent baseline separation for chloride, phosphate and sulfate.

Conclusion

The analytical challenge treated in the present work consisted in the determination of chloride, phosphate and sulfate in the presence of difficult sample matrices that could interact with the stationary column phase or even render it unusable. A patented stopped-flow dialysis system coupled to the new 881 Compact IC pro ion chromatograph overcomes these drawbacks.

Two standard solutions covering the concentration ranges 1.0 to 3.6 mg/L and 10 to 36 mg/L as well as two samples: an ultra-high temperature (UHT) processed milk and a baby milk powder, were characterised in terms of analyte concentration, relative standard deviation, calibration quality, carryover and recovery rates. The five-point calibration curves yielded correlation coefficients (R) better than 0.9999 and carryover between two subsequent injections of a concentrated sample and a blank was found to be less than 0.49%. Recoveries for the low (1.0 to 3.6 mg/L) and high standard concentration ranges (10 to 36 mg/L) were within 91 to 99% and 94 to 100%, respectively.

Automated compact stopped-flow dialysis is a highly efficient sample preparation technique that ensures optimum separation performance by protecting the column from detrimental matrix constituents.

	Fluoride	Chloride	Nitrite	Bromide	Nitrate	Phosphate	Sulfate
Correlation coefficient	0.99995	0.99996	0.99999	0.99996	0.99994	0.99990	0.99997
RSD [%]	1.516	1.242	0.834	1.169	1.479	2.491	1.176

Table 1: Correlation coefficients and relative standard deviations of the five-point anion calibration

	Fluoride	Chloride	Nitrite	Bromide	Nitrate	Phosphate	Sulfate
Low standard conc.	0.24	0.15	0.17	0.20	0.18	0.11	0.28
High standard conc.	0.49	0.12	0.13	0.22	0.11	0.00	0.38

Table 2: Carryover in percent determined for the concentration ranges 1.0…3.6 mg/L and 10…36 mg/L

	Low standard concentration (1.0…3.6 mg/L)					High standard concentration (10…36 mg/L)				
	Direct injection		With dialysis		Recovery	Direct injection		With dialysis		Recovery
	Mean [mg/L]	RSD [%]	Mean [mg/L]	RSD [%]	rate [%]	Mean [mg/L]	RSD [%]	Mean [mg/L]	RSD [%]	rate [%]
Fluoride	1.06	0.12	1.03	0.24	**97.2**	10.81	0.09	10.57	0.06	**97.8**
Chloride	3.01	0.04	2.97	0.03	**98.7**	31.58	0.03	31.22	0.06	**98.9**
Nitrite	2.94	0.32	2.91	0.15	**99.0**	30.01	0.30	29.81	0.04	**99.3**
Bromide	1.02	0.08	1.01	0.00	**99.0**	10.50	0.04	10.38	0.17	**98.9**
Nitrate	3.02	0.07	2.97	0.00	**98.3**	30.80	0.03	30.40	0.03	**98.7**
Phosphate	3.81	0.17	3.47	0.10	**91.1**	33.74	0.02	31.83	0.03	**94.3**
Sulfate	3.52	0.09	3.35	0.07	**95.2**	35.57	0.04	34.17	0.07	**96.1**

Table 3: Anion recovery rates

References

1. Metrohm Application Notes AN-S-044, AN-S-162, AN-N-018, AN-C-100 and AN-C-028 (downloadable under http://products.metrohm.com).
2. Metrohm Monograph: Sample preparation techniques for ion chromatography, Metrohm AG, Herisau, Switzerland, 108 pages, 8.025.5003.
3. Steinbach A. and Wille A., Ion chromatographic analysis of carbohydrates in essential and non-essential foodstuffs, *Food Engineering & Ingredients*, 33-36 (2008).

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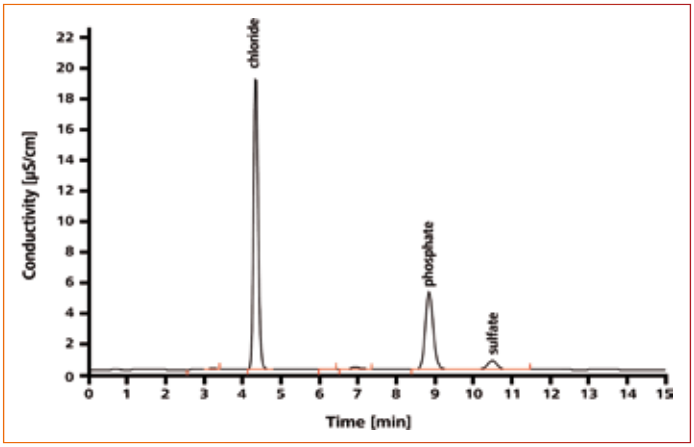

Figure 3: Anion chromatogram of a UHT dialysate containing 9.88 mg/L chloride, 17.40 mg/L phosphate and 1.09 mg/L sulfate (after 1:100 dilution of the sample). Column: Metrosep A Supp 5 - 100/4.0, eluent: 3.2 mmol/L sodium carbonate and 1.0 mmol/L sodium hydrogen carbonate, flow: 0.7 mL/min, column temperature: 30 °C, injection volume: 20 µL, acceptor solution: ultrapure water, dialysis time: 14 min.

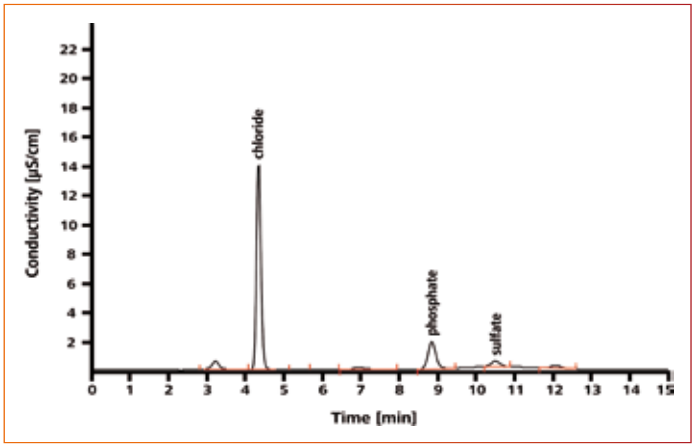

Figure 4: Anion chromatogram of a baby food milk sample containing 7.37 mg/L chloride, 7.41 mg/L phosphate and 0.76 mg/L sulfate (after 1:100 dilution of the sample). Chromatographic conditions correspond to those given in Figure 3.