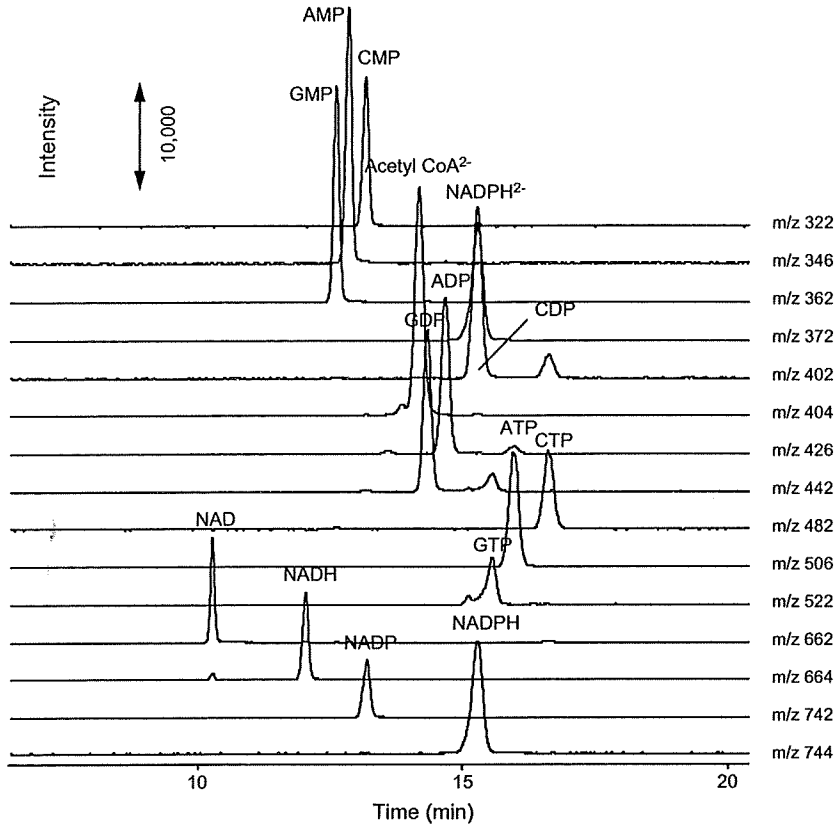

Fig. 4. PACE–MS selected ion electropherograms for standard mixture (100 μM each) of nucleotides, nicotinamide–adenine dinucleotides and CoA compounds. Experimental conditions: nebulizer gas pressure, 10 psig after 0.1 min. Other experimental conditions are the same as in Fig. 3.

Table 2
Nucleotide concentration of *E. coli* BW25113 wild type, Δ*pfkA* and Δ*pfkB* knockout mutant

Compound	Wild type			Δ*pfkA*	Δ*pfkB*
	Concentration (μM)	RSD ($n=35$) (%)	Recovery rate ($n=3$)(%)	Concentration (μM)	Concentration (μM)
TMP	693	2.9	95.7 ± 4.8	91	38
cAMP	10	19	93.1 ± 2.5	2	9
cGMP	nd[a]	–	94.5 ± 3.1	19	nd[a]
AMP	890	2.8	100.0 ± 5.8	547	1060
GMP	81	4.2	94.5 ± 4.5	73	99
CoA	30	28	90.4 ± 1.6	94	66
TDP	1260	4.0	95.8 ± 3.1	728	64
CDP	338	3.6	97.7 ± 3.4	256	179
Acetyl CoA	358	2.5	93.8 ± 4.2	74	127
ADP	1427	5.0	94.3 ± 4.8	340	670
Succinyl CoA	nd[a]	–	95.6 ± 2.9	6	nd
GDP	439	5.1	95.8 ± 5.1	146	248
TTP	148	5.7	93.2 ± 6.0	28	133
CTP	324	4.6	92.9 ± 5.1	111	320
ATP	1640	5.7	97.6 ± 3.8	239	1560
GTP	696	4.9	92.0 ± 5.2	95	680
NAD	1200	2.9	101.7 ± 2.7	391	941
NADH	nd[a]	–	83.7 ± 4.5	nd[a]	nd[a]
NADP	209	5.1	92.2 ± 2.1	39	162
NADPH	nd[a]	–	70.1 ± 5.3	nd[a]	nd[a]
FAD	16	8.6	95.4 ± 4.5	8	55

[a] nd; not detected.

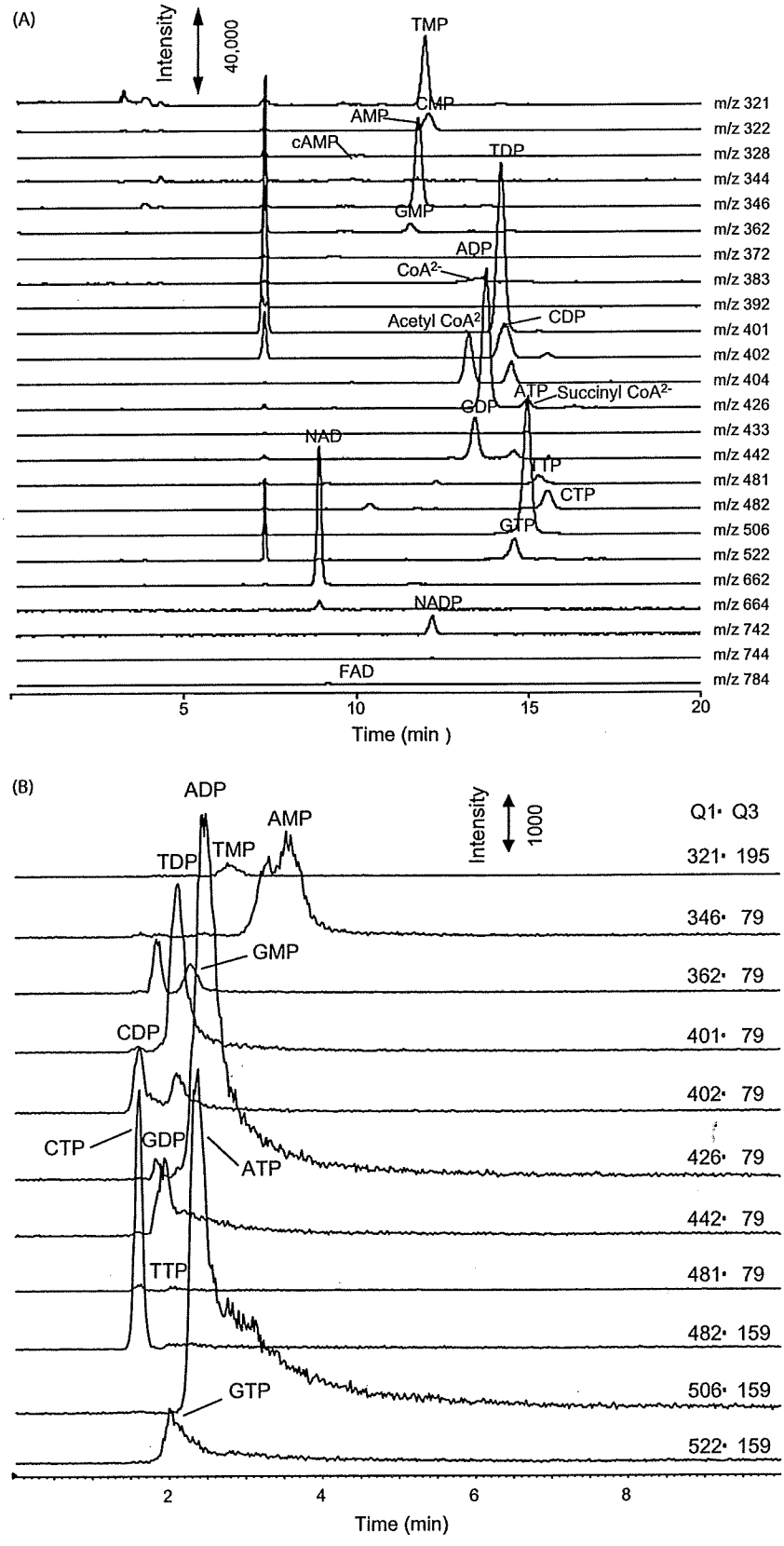

Fig. 5. Comparison of (A) PACE–MS and (B) LC–MS/MS analysis of nucleotides, nicotinamide–adenine dinucleotides and CoA compounds in the extract from *E. coli* BW25113 wild type. Experimental conditions of PACE-MS: the same as in Fig. 4. For conditions of LC–MS/MS, see Section 2. The optimized parameters, Q1 (precursor ion), Q3 (product ion), declustering potential, focusing potential, collision energy and collision cell exit potential are listed in Table 3.

Table 3
Optimized MRM parameters for each nucleotide in LC–MS/MS

Nucleotide	Q1 (*m/z*)	Q3 (*m/z*)	Declustering potential (V)	Focusing potential (V)	Collision energy (V)	Collision cell exit potential (V)
TMP	321	195	−52	−82	−22	−6
AMP	346	79	−58	−103	−52	−4
GMP	362	79	−45	−90	−38	−5
TDP	401	79	−48	−86	−59	−5
CDP	402	79	−50	−170	−58	−3
ADP	426	79	−41	−90	−60	−5
GDP	442	79	−50	−100	−68	−5
TTP	481	159	−46	−100	−40	−9
CTP	482	159	−58	−160	−34	−6
ATP	506	159	−41	−104	−42	−7
GTP	522	159	−54	−177	−45	−8

of nucleotide and CoA standards and were quantified (Table 2). The relative standard deviations ($n = 35$) for the amounts of identified compounds in the sample were better than 6% except for small compounds. Under these conditions, however, neither NADH nor reduced nicotinamide–adenine dinucleotide phosphate (NADPH) could be observed. To investigate quantification accuracy and ion suppression effect in this system, we analyzed cell extracts of wild type *E. coli* BW25113 spiked with 100 μM of each standard and calculated the recovery (Table 2) and compared with the results obtained by the LC–MS/MS method (Fig. 5B). Except for nicotinamide–adenine dinucleotide derivatives (NADPH/$NADP^+$), the recovery rates of metabolites in the PACE–MS were higher than 90%. Surprisingly, the recovery rates in LC–MS/MS were quite low. Those of TMP, AMP, GMP, TDP, CDP, ADP, GDP, TTP, CTP, ATP and GTP in LC–MS/MS were 62, 25, 40, 43, 25, 31, 27, 21, 37, 23 and 19%, respectively. Considering the good linearity of calibration curves in the concentration ranges of the LC–MS/MS method, these poor recovery rates may be caused by ion suppression effect because all nucleotides were retained poorly (Fig. 5B) and eluted together with other charged components in *E. coli* extracts. On the other hand, the PACE–MS provides much better resolution for nucleotides compared to LC–MS/MS. In addition, the injection volume in the PACE–MS (30 nL) is considerably small compared to that in LC–MS/MS (1 μL). Therefore, the PACE–MS seems to be scarcely affected by ion suppression effect and it enables sufficient quantitative analysis for most nucleotides and CoA compounds. As the conversion of NADH to NAD or NADPH to NADP occurs depending on physical conditions such as sample pH and temperature, further examination for sample treatment is required to quantify them correctly (Table 3).

We also analyzed nucleotide and CoA related compounds in the knockout mutant of *E. coli* BW25113 *pfkA* or *pfkB*, the isoenzyme that catalyzes the production of fructose 1,6-bisphophate from fructose 6-phosphate in glycolytic pathway. The results were compared with *E. coli* BW25113 wild type (Table 2) and distinct amount differences in many compounds between samples were observed. It was found that considerable decrease in the acetyl CoA, ADP, ATP, NAD, NADH and FAD levels in the *pfkA* mutant compared to those in the *pfkB* mutant, which may suggest that deletion of *pfkA* had significant effect of metabolism of glycolytic and the TCA pathway. This hypothesis that *pfkA* can be the dominant enzyme in *E. coli*, is supported by a previous study [31].

4. Conclusions

A new pressure-assisted CE–MS method for the analysis of nucleotides has been developed. Compared with other techniques, this method has several advantages: (1) various types of phosphorylated species such as nucleotides, nicotinamide–adenine dinucleotides and CoA compounds are simultaneously analyzed; (2) sensitivity and selectively are sufficient for quantification; and (3) good reproducibility, linearity and robustness are obtained. Its utility was demonstrated by the analysis of these phosphorylated species in *E. coli* wild type, *pfkA* or *pfkB* knockout mutant and the results can potentially provide valuable information about the enzyme activity. The PACE–MS method developed in this study can be a promising new tool for the analysis of nucleotides, nicotinamide–adenine dinucleotides and CoA compounds in biological samples.

Acknowledgments

We thank Dr. Kenji Nakahigasi, Dr. Takashi Hirasawa, Miki Naba and Kenta Hirai, Institute for Advanced Biosciences, Keio University, for technical support and Dr. David N. Heiger, Agilent Technologies, for critical reading of the manuscript. This work was supported in part by a grant the 21st Century COE Program entitled "Understanding and Control of Life's Function via Systems Biology" and a grant-in-aid for scientific research on priority areas "Lifesurveyor" from the Ministry of Education, Culture, Sport, Science and Technology (MEXT) of Japan as well as research funds from Yamagata prefectural government and Tsuruoka city.

References

[1] O. Fiehn, J. Kopka, P. Dormann, T. Altmann, R.N. Trethewey, L. Willmitzer, Nat. Biotechnol. 18 (2000) 1157.
[2] N. Schauer, Y. Semel, U. Roessner, A. Gur, I. Balbo, F. Carrari, T. Pleban, A. Perez-Melis, C. Bruedigam, J. Kopka, L. Willmitzer, D. Zamir, A.R. Fernie, Nat. Biotechnol. 24 (2006) 447.

[3] R. Plumb, J. Granger, C. Stumpf, I.D. Wilson, J.A. Evans, E.M. Lenz, Analyst 128 (2003) 819.
[4] N.V. Reo, Drug Chem. Toxicol. 25 (2002) 375.
[5] M. Coen, E.M. Lenz, J.K. Nicholson, I.D. Wilson, F. Pognan, J.C. Lindon, Chem. Res. Toxicol. 16 (2003) 295.
[6] M. Coen, S.U. Ruepp, J.C. Lindon, J.K. Nicholson, F. Pognan, E.M. Lenz, I.D. Wilson, J. Pharm. Biomed. Anal. 35 (2004) 93.
[7] A. Aharoni, C.H. Ric de Vos, H.A. Verhoeven, C.A. Maliepaard, G. Kruppa, R. Bino, D.B. Goodenowe, Omics 6 (2002) 217.
[8] M.Y. Hirai, M. Yano, D.B. Goodenowe, S. Kanaya, T. Kimura, M. Awazuhara, M. Arita, T. Fujiwara, K. Saito, Proc. Natl. Acad. Sci. USA 101 (2004) 10205.
[9] S.K. Johnson, L.L. Houk, D.C. Johnson, R.S. Houk, Anal. Chim. Acta 389 (1999) 1.
[10] W. Lu, G.K. Poon, P.L. Carmichael, R.B. Cole, Anal. Chem. 68 (1996) 668.
[11] M. Moini, Methods Mol. Biol. 276 (2004) 253.
[12] T. Soga, D.N. Heiger, Anal. Chem. 72 (2000) 1236.
[13] T. Soga, Y. Ueno, H. Naraoka, Y. Ohashi, M. Tomita, T. Nishioka, Anal. Chem. 74 (2002) 2233.
[14] C.C. Liu, J.S. Huang, D.L. Tyrrell, N.J. Dovichi, Electrophoresis 26 (2005) 1424.
[15] T. Soga, Y. Ohashi, Y. Ueno, H. Naraoka, M. Tomita, T. Nishioka, J. Proteome Res. 2 (2003) 488.
[16] S. Sato, T. Soga, T. Nishioka, M. Tomita, Plant J. 40 (2004) 151.
[17] T. Soga, R. Baran, M. Suematsu, Y. Ueno, S. Ikeda, T. Sakurakawa, Y. Kakazu, T. Ishikawa, M. Robert, T. Nishioka, M. Tomita, J. Biol. Chem. 281 (2006) 16768.
[18] T. Soga, Y. Ueno, H. Naraoka, K. Matsuda, M. Tomita, T. Nishioka, Anal. Chem. 74 (2002) 6224.
[19] P. Cao, M. Moini, Electrophoresis 19 (1998) 2200.
[20] K.D. Lukacs, J.W. Jorgenson, J. High Res. Chromatogr. 8 (1985) 407.
[21] T. Baba, T. Ara, M. Hasegawa, Y. Takai, Y. Okumura, M. Baba, K.A. Datsenko, M. Tomita, B.L. Wanner, H. Mori, Mol. Syst. Biol. (2006), doi:10.1038/msb4100050.
[22] J. Zhao, T. Baba, H. Mori, K. Shimizu, Metab. Eng. 6 (2004) 164.
[23] N.J. Oppenheimer, in: D. Dolphin, R. Poulson, O. Avramovic (Eds.), Pyridine Nucleotide Coenzymes Part B (Coenzymes and Cofactors), 2, John Wiley & Sons, New York, NY, 1987, p. 323.
[24] N.O. Kaplan, S.P. Colowick, C.C. Barnes, J. Biol. Chem. 191 (1951) 461.
[25] Q. Liu, F. Lin, R.A. Hartwick, Chromatographia 47 (1998) 219.
[26] G. Liu, Chromatographia 28 (1989) 493.
[27] M.L. Hair, W. Hertl, J. Phys. Chem. 74 (1970) 91.
[28] J. Kim, D.G. Camp II, R.D. Smith, J. Mass Spectrom. 39 (2004) 208.
[29] F. Benavente, E. Balaguer, J. Barbosa, V. Sanz-Nebot, J. Chromatogr. A 1117 (2006) 94.
[30] M. Sugimoto, S. Kikuchi, M. Arita, T. Soga, T. Nishioka, M. Tomita, Anal. Chem. 77 (2005) 78.
[31] J. Babul, J. Biol. Chem. 253 (1978) 4350.

Anal. Chem. **2007**, *79*, 7838–7844

Polymer Entrapment in Polymerized Silicate for Preparing Highly Stable Capillary Coatings for CE and CE–MS

Maria Rowena N. Monton,† Masaru Tomita,† Tomoyoshi Soga,*,† and Yasushi Ishihama*,†,‡

Institute for Advanced Biosciences, Keio University, Tsuruoka, Yamagata 997-0017, Japan, and PRESTO, Japan Science and Technology Agency, Sanbancho Building, 5-Sanbancho, Chiyodaku, Tokyo 102-0075, Japan

An easy-to-implement capillary coating strategy based on polymer entrapment in the network of polymerized silicate is described. In this manner, cationic polymers are tightly fixed onto the inner wall of the capillary for electroosmotic flow control without necessitating complex surface modification chemistries. The resulting coated capillary exhibited good stability over a wide range of pH, good reproducibility, strong endurance in more than 300 electrophoretic runs, and tolerance of commonly employed organic solvent additives in CE. Applications in CE–MS analysis of biologically important anions as well as sample enrichment are shown. Additionally, it was used as a durable base for attachment of multiple layers of charged polymers on the wall, via electrostatic interaction with the preceding layer. Thus, two novel types of highly stable coated capillaries, one with anodic EOF and the other cathodic, were developed.

Capillary electrophoresis (CE) has always been touted as a high-efficiency separation technique, but its potential is seldom fully realized because analyte–wall interactions, compounded by high surface area-to-volume ratios of narrow-bore capillaries, result in some band broadening. Moreover, migration time shifts pose a major problem in CE systems, which rely on matching peak migration times against those of pure standards for identification. The intrinsic electrophoretic mobility of an analyte is constant under a given set of conditions, but its apparent mobility is often less reproducible because of run-to-run variations in electroosmotic flow (EOF).[1] The EOF is a consequence of electroneutrality constraint on the wall. Depending on the separation problem, its direction may be reversed, or its magnitude augmented or diminished, or it may be completely eliminated. Thus, it is important to control surface chemistry. Numerous approaches have been explored for regulating wall properties of fused-silica capillaries, including manipulation of pH,[2] ionic strength,[3] and viscosity[4] of background electrolytes, but the most common strategy is to coat the inner wall of the capillary.

Based on the attachment of the coating onto the wall, coatings are broadly categorized as physically adsorbed or covalently attached.[5] Physically adsorbed coatings result from reversible adsorption of charged (e.g., Polybrene (PB),[6,7] polyethyleneimine (PEI),[8] poly(dimethyldiallylammonium chloride),[9] dextran sulfate (DS),[10] polyarginine[11]) or neutral (e.g., poly(ethylene oxide),[12,13] poly(vinyl alcohol),[14] hydroxyethyl cellulose[15]) polymeric or small-molecule (e.g., cetyltrimethylammonium bromide,[16] didodecyldimethylammonium bromide,[17] 1,2-dilauroyl-*sn*-phosphatidylcholine,[18] triethylenetetramine,[19] trimethylammoniopropane sulfonate,[20] 1,2-dioleyl-3-trimethylammoniumpropane[21]) additives on the wall via hydrogen bonding or electrostatic interactions. The coating procedures are fairly simple and generally entail only rinsing the capillary with a dilute aqueous solution of the additive. The working lifetimes of coated capillaries can be easily extended by regeneration, i.e., by rinsing out the additive, and then replacing it with a fresh layer of the same. Physically adsorbed coatings can be either dynamic or static.[5] To compensate for the poor stability of dynamic adsorbed coatings, the additive must be incorporated in the running buffer, and in some cases, this can

* To whom correspondence should be addressed. E-mail:soga@sfc.keio.ac.jp. Tel: +81-235-29-0528. Fax: +81-235-29-0574. E-mail: y-ishi@ttck.keio.ac.jp. Tel.: +81-235-29-0571. Fax: +81-235-29-0536.

† Keio University.

‡ PRESTO, Japan Science and Technology Agency.

(1) Chiari, M.; Cretich, M.; Horvath, J. *Electrophoresis* **2000**, *21*, 1521–1526.
(2) Hayes, M. A.; Kheterpal, I.; Ewing, A. *Anal. Chem.* **1993**, *65*, 27–31.
(3) Green, J. S.; Jorgenson, J. W. *J. Chromatogr.* **1989**, *478*, 63–70.
(4) Schwer, C.; Kenndler, E. *Anal. Chem.* **1991**, *63*, 1801–1807.
(5) Doherty, E. A. S.; Meagher, R. J.; Albarghouti, M. N.; Barron, A. E. *Electrophoresis* **2003**, *24*, 34–54.
(6) Masselter, S. M.; Zemann, A. J. *Anal. Chem.* **1995**, *67*, 1047–1053.
(7) Li, M. X.; Liu, L.; Wu, J.-T.; Lubman, D. M. *Anal. Chem.* **1997**, *69*, 2451–2456.
(8) Erim, F. B.; Cifuentes, A.; Poppe, H.; Kraak, J. C. *J. Chromatogr., A* **1995**, *708*, 356–361.
(9) Wang, Y.; Dubin, P. L. *Anal. Chem.* **1999**, *71*, 3463–3468.
(10) Katayama, H.; Ishihama, Y.; Asakawa, N. *Anal. Chem.* **1998**, *70*, 2254–2260.
(11) Chiu, R. W.; Jimenez, J. C.; Monnig, C. A. *Anal. Chim. Acta* **1995**, *307*, 193–201.
(12) Iki, N.; Yeung, E. S. *J. Chromatogr., A* **1996**, *731*, 273–282.
(13) Vayaboury, W.; Kirby, D.; Giani, O.; Cottet, H. *Electrophoresis* **2005**, *26*, 2187–2197.
(14) Gilges, M.; Kleemiss, M. H.; Schomburg, G. *Anal. Chem.* **1994**, *66*, 2038–2046.
(15) Kleemiss, M. H.; Gilges, M.; Schomburg, G. *Electrophoresis* **1993**, *14*, 515–522.
(16) Reijenga, J. C.; Aben, G. V. A.; Verheggen, T. P. E. M.; Everaerts, F. M. *J. Chromatogr.* **1983**, *260*, 241–254.
(17) Melanson, J. E.; Baryla, N. E.; Lucy, C. A. *Anal. Chem.* **2000**, *72*, 4110–4114.
(18) Baryla, N. E.; Lucy, C. A. *J. Chromatogr., A* **2002**, *956*, 271–277.
(19) Corradini, D.; Sprecacenere, L. *Chromatographia* **2003**, *58*, 587–596.
(20) MacDonald, A. M.; Sheppard, M. A. W.; Lucy, C. A. *Electrophoresis* **2005**, *26*, 4421–4428.
(21) Bonoli, M.; Varjo, S. J. O.; Wiedmer, S. K.; Riekkola, M.-L. *J. Chromatogr., A* **2006**, *1119*, 163–169.

10.1021/ac071038y CCC: $37.00
Published on Web 09/08/2007

cause unwanted interactions with the analytes or interference with the detection scheme (e.g., mass spectrometry (MS)). In contrast, static adsorbed coatings, including the so-called semipermanent coatings[17,18,21] and those formed from alternating layers of oppositely charged polymers,[10,22,23] are sufficiently stable and therefore do not require the presence of the additive in the running buffer. By covalent bonding, polymers (e.g., polyacrylamide,[24-27] poly(vinylpyrrolidone),[28] poly(ethylene glycol)[29]) and small molecules (e.g., octadecylsilane)[30] can also be fixed to the wall. In most cases, the capillary surface is initially derivatized with bifunctional reagents to provide anchors[5] through which polymers, either polymerized in situ or preformed, and then subsequently cross-linked to increase surface coverage, are attached to the wall. Coated capillaries prepared using covalent bonding generally exhibit longer working lifetimes than those prepared using physical adsorption method, but the complexity and multiplicity of steps required in the preparation of the former often lead to poor coating-to-coating reproducibility

Soluble silicates of sodium and potassium have a long history of use in a wide range of bonding and coating applications, and when applied as thin films on or between surfaces, they dry to form tough, tightly adhering inorganic coatings or bonds.[31] To date, polymerized silicates have been exploited in a number of applications for separation science, such as for gluing chromatographic beads together for capillary electrochromatography,[32] for preparing monolithic columns for liquid chromatography,[33] and for immobilizing enzymes at the bottom of a 96-well microtiter plate.[34]

In this paper, we describe a novel way of modifying the inner wall of the capillary based on the immobilization of a positively charged polymer by entrapment in a matrix of polymerized silicate. This technique combines the simplicity in terms of preparation of physically adsorbed coatings with the stability generally characteristic of covalently attached ones. The basic properties of the coated capillary are discussed, and some applications are shown.

EXPERIMENTAL SECTION

Reagents. Potassium silicate solution (Kasil 1) was a gift from PQ Corp. (Valley Forge, PA). Tryptic digest of pig serum albumin was from Michrom Bioresources (Auburn, CA). PB, diethylaminoethyl (DEAE)-dextran, PEI, DS, hexamethyldisilazane (HMDS), horse heart myoglobin, trypsin inhibitor, β-lactoglobulin A, dihydroxyacetone phosphate, glycerol 3-phosphate, 2-phosphoglycerate, 2,3-diphosphoglycerate, 6-phosphogluconate, and erythrose 4-phosphate were from Sigma (St. Louis, MO). All other reagents were obtained from Wako (Osaka, Japan). Buffers and standard solutions were prepared using water purified with a Milli-Q system from Millipore (Bedford, MA), or aqueous–alcoholic solutions.

Instruments. CE experiments were performed on an Agilent CE system (Waldbronn, Germany) using fused-silica capillaries (50 μm i.d. × 360 μm o.d.) from Polymicro Technologies (Phoenix, AZ), uncoated or coated in-house. The capillaries were thermostated at 25 °C. Unless specified otherwise, samples were injected by applying a 50-mbar pressure at the inlet vial for 3 s. EOF mobilities were measured using formamide as electrically neutral marker. Using UV detection, absorbance values were monitored at 200 nm. For CE–MS experiments, the CE instrument was coupled to an Agilent 1100 series MSD mass spectrometer, via a sheath liquid-assisted electrospray ionization (ESI) interface. ESI-MS was conducted in the negative ion mode, with the capillary voltage set at 3500 V. A flow of heated dry nitrogen gas was maintained at 10 L/min. The spectrometer was operated in the selected ion monitoring mode. Other conditions are indicated in relevant text or figures.

All capillary cleaning and coating procedures were carried out at room temperature using Terumo disposable syringes (Tokyo, Japan) and a KD Scientific syringe pump (Holliston, MA). pH measurements and adjustments were made using a Horiba F22 pH meter (Kyoto, Japan). The conductivities of buffer solutions (phosphate, pH 2–3, 6–7; acetate, pH 4–5; borate, pH 8–11) used in EOF versus pH experiments were measured and adjusted to be approximately equal using a Horiba ES-51 conductivity meter (Kyoto, Japan).

Capillary Coating. The new capillary was flushed successively with 1 M sodium hydroxide and water for 30 min and then purged with a stream of nitrogen gas for 5 min to dry the surface. The 200 μL of potassium silicate solution (Kasil 1), 200 μL of water, 40 μL of formamide, and 80 μL of aqueous cationic polymer solution were mixed thoroughly, and the resulting mixture was pumped into the capillary (10 min for a 50-cm capillary; 20 min for a 120-cm capillary) at room temperature. After 1 h, the excess coating solution was removed by air-flushing, and the coated capillary was allowed to dry overnight. For multilayer coatings, the preceding drying step was omitted; instead, an aqueous solution of the oppositely charged polymer was rinsed into the capillary (10 min for a 50-cm capillary; 20 min for a 120-cm capillary), allowed to stand for 30 min, and the excess was removed and then either left to dry or the next layer was applied. The concentrations of polymers used in the coatings are indicated in relevant text and figures as % w/v.

RESULTS AND DISCUSSION

Polymerized Silicate-Entrapped Polymer for Capillary Coating. The present coating technique based on polymerized silicate can be likened to laying an additional layer of glass on the inner wall of the capillary, except that this glass layer contains polymeric additives to alter the surface charge (Figure 1A). In this work, potassium silicate (Kasil 1) was polymerized via a sol–gel process, using formamide as drying control chemical additive to increase the mechanical strength of the gel,[35] and mixed with

(22) Katayama, H.; Ishihama, Y.; Asakawa, N. *Anal. Chem.* **1998**, *70*, 5272–5277.
(23) Graul, T. W.; Schlenoff, J. B. *Anal. Chem.* **1999**, *71*, 4007–4013.
(24) Hjerten, S. *J. Chromatogr.* **1985**, *347*, 191–198.
(25) Cobb, K. A.; Dolnik, V.; Novotny, M. *Anal. Chem.* **1990**, *62*, 2478–2483.
(26) Cifuentes, A.; Canajales, P.; Diez-Masa, J. C. *J. Chromatogr., A* **1999**, *830*, 423–438.
(27) Liu, S.; Gao, L.; Pu, Q.; Lu, J. J.; Wang, X. *J. Proteome Res.* **2006**, *5*, 323–329.
(28) Srinivasan, K.; Pohl, C.; Avladovic, N. *Anal. Chem.* **1997**, *69*, 2798–2805.
(29) Razunguzwa, T. T.; Warrier, M.; Timperman, A. T. *Anal. Chem.* **2006**, *78*, 4326–4333.
(30) Towns, J. K.; Regnier, F. E. *Anal. Chem.* **1991**, *63*, 1126–1132.
(31) PQ Corp., Bulletin 12–31, 2003.
(32) Chirica, G.; Remcho, V. T. *Electrophoresis* **1999**, *20*, 50–56.
(33) Fields, S. M. *Anal. Chem.* **1996**, *68*, 2709–2712.
(34) Sakai-Kato, K.; Kato, M.; Homma, H.; Toyo'oka, T.; Utsunomiya-Tate, N. *Anal. Chem.* **2005**, *77*, 7080–7083.
(35) Lenza, R. F. S.; Vasconcelos, W. L. *Mater. Res.* **2001**, *4*, 175–179.

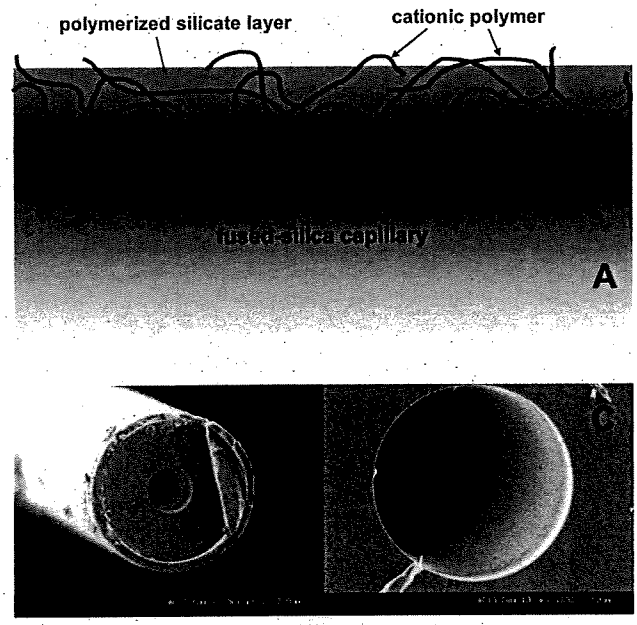

Figure 1. Schematic representation of cationic polymer entrapment in polymerized silicate (A). Scanning electron micrographs of the coated capillary (B and C). Capillary, 50 μm i.d. × 360 μm o.d.; coating, 1.5% PB in the coating mixture.

cationic polymers, such as PB. After the resulting mixture was pumped into the capillary, the excess removed and then allowed to dry, it yielded a stable coating, which we have named as KEIO (*K*asil-entrapped *io*nic) polymer coating. Scanning electron micrographs showed that the coated layer was between 1.2 and 1.4 μm in thickness (Figure 1B), and a "clean" surface was produced (Figure 1C). In X-ray diffraction studies of the structure of glass,[36] each silicon atom is tetrahedrally surrounded by four oxygen atoms, and part of the oxygen is bonded between two silicon and part to only one silicon. Although there is a distinct organization in the structure, there is no regular repetition in the pattern, resulting in a random silicon–oxygen network. The cationic polymer can be presumed to be entrapped within the various holes of the network.

Characterization of the KEIO–PB Coating. (1) Effect of Polymer Concentration on EOF Mobility. Similar to bare silica, the inner wall of the KEIO polymer-coated capillary intrinsically possesses negatively charged sites because of the presence of ionizable silanol groups. Incorporation of a positively charged polymer in the coated layer diminishes the net negative charge of the surface, which becomes progressively lower as the concentration of the polymer increases. At a polymer concentration that is high enough to supersede the effect of the silanols, the net charge of the surface becomes positive. The positive charge steadily increases as more polymers are incorporated until such point where further increases in polymer concentration no longer affect the resulting charge significantly.

Changes in surface charge will be reflected in the EOF. Figure 2 tracks the EOF as a function of the concentration of the cationic polymer PB in the coating mixture. The measurements were performed at pH 7, in which ionization of silanols was evident as well. In the absence of PB in the coating (i.e, a mixture of Kasil 1, water, and formamide only), a fast, normal (i.e., from anode to cathode) EOF was generated. At low PB concentrations, the EOF remained cathode-directed, albeit becoming slower as the concentration of PB increased. At 0.1%, the EOF was reversed and then became faster with addition of more PB to the coating. At ~0.3%, the magnitude of the reversed EOF leveled off and was no longer significantly affected by further increases in PB concentration.

These results suggest that it is possible to manipulate the EOF in terms of both direction and magnitude by simply adjusting the concentration of the polymer in the coating. Thus, using KEIO–PB, the EOF can be tuned easily as may be required by the separation problem. In contrast, prior to commercialization of coating systems like CEofix[37] and EOtrol,[38] it is generally difficult to control the EOF in capillaries coated by conventional physical adsorption.[39]

(2) Effect of Buffer pH on EOF Mobility. The variation of EOF with pH under constant ionic strength conditions is represented graphically in Figure 3. In an uncoated capillary, the velocity of a cathodic EOF was clearly pH-dependent, i.e., high under neutral or alkaline conditions, reduced below pH 6, and significantly suppressed below pH 3. On the other hand, in a KEIO–PB(1.5%) capillary, a strong, anodic EOF was generated throughout the pH range 2–11, although it was slightly faster in the acidic region, where the ionization of silanols, which exert an effect counter to PB, was suppressed. In this manner, the pH can be varied to modify selectivity without considerably affecting the flow rate.

(3) Stability. The stability of KEIO–PB(1.5%) capillary was evaluated by monitoring the EOF in 300 successive runs, without any capillary conditioning except a 3-min high-pressure (~940 mbar) rinse with the running buffer between consecutive analyses. Detachment of PB from the polymerized silicate is expected to be manifested as slowing down of the reversed EOF. The evaluation was performed at three pH values (pH 2.5, 4.5, and 8.5), and the results are shown in Figure 4. At pH 2.5 and 4.5, the EOF was practically unchanged over the 300-run course, thereby demonstrating the strong endurance of the coated capillary under acidic conditions. At pH 8.5, the EOF was initially slow and appeared to degrade faster, which could be attributed to the intrinsic instability of silicates at alkaline conditions. Regardless, the degradation, calculated to be ~22% at the completion of the 300th run, could still be considered reasonable, and stability was further improved by HMDS treatment of the coated capillary to protect the silanols prior to use (data not shown). While silicate itself is a strong adhesive and binder, which attaches the polymers tightly onto the wall, the long working lifetime of the coated capillary can also be attributed to the presence of polymeric additives all throughout the coating and not just on the surface. Thus, even if the outermost sections of the coating become unraveled from use, there are still polymers remaining in the next exposed level.

Addition of organic solvents to running buffers often expands the range of application of CE. Aqueous–organic solvent mixtures

(36) Warren, B. E.; Biscoe, J. *J. Am. Ceram. Soc.* **1938**, *21*, 259–265.

(37) Lanz, C.; Marti, U.; Thormann, W. *J. Chromatogr., A* **2003**, *1013*, 131–147.

(38) Chang, W. P.; Nichols, L.; Jiang, K.; Schneider, L. V. *Am. Lab. News* **2004**, *36*, 8–13.

(39) Katayama, H.; Ishihama, Y.; Asakawa, N. *J. Chromatogr., A* **2000**, *875*, 315–322.

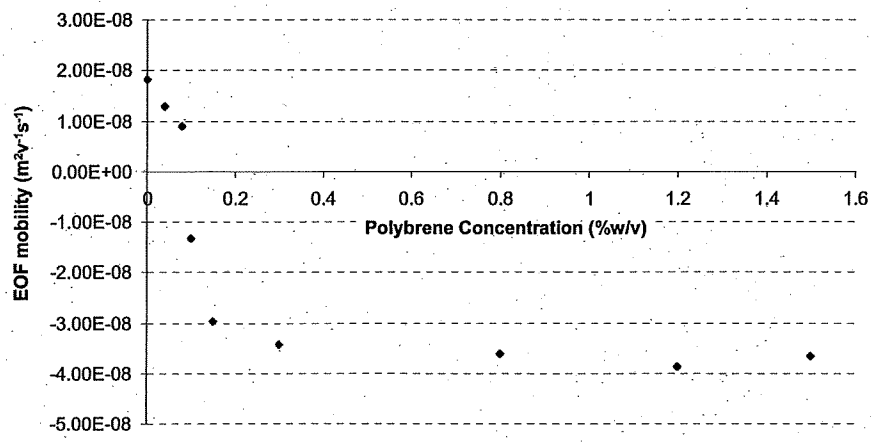

Figure 2. Dependence of EOF on concentration of PB in KEIO–PB capillaries. Conditions: capillary, 38.5 cm (30-cm effective length); background solution (BGS), 50 mM ammonium acetate, pH 7.0; applied voltage, ±20 kV.

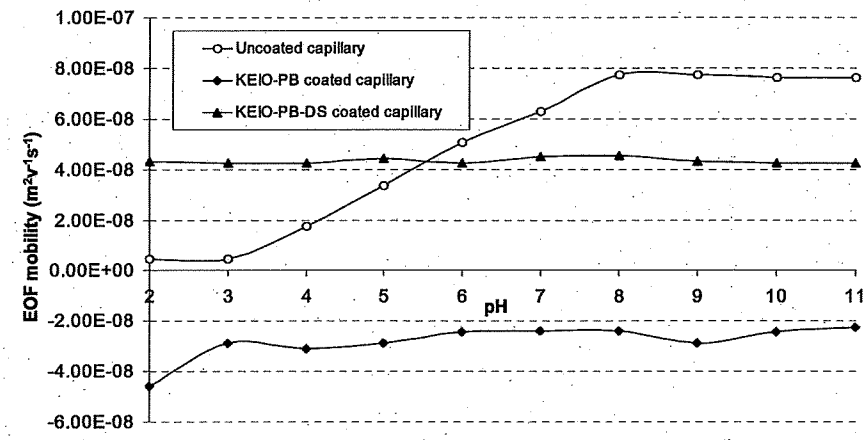

Figure 3. EOF vs pH profiles of uncoated, KEIO–PB(1.5%), and KEIO–PB(1.5%)–DS(3%) capillaries. Conditions: capillary, 38.5 cm (30 cm effective length); applied voltage, ±30 kV.

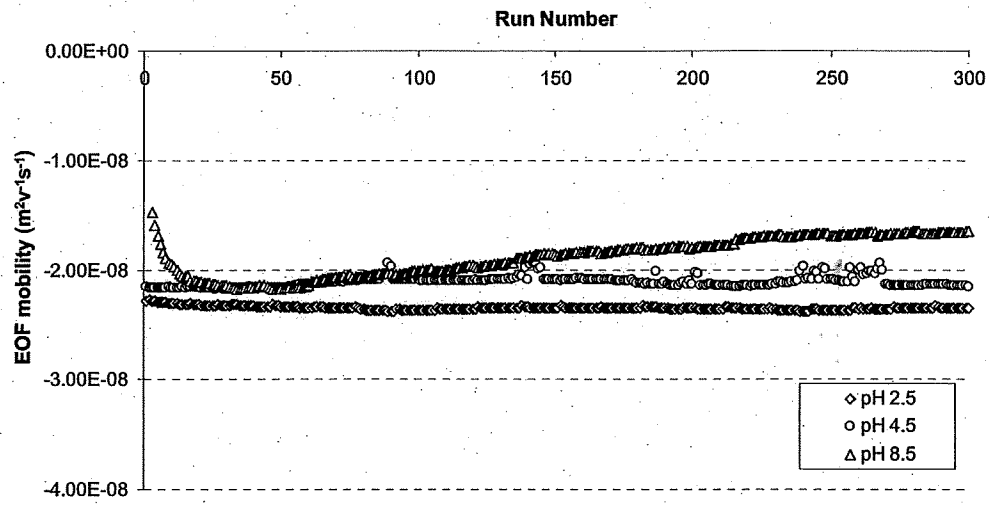

Figure 4. Endurance of KEIO–PB(1.5%) capillary. Conditions: capillary, 38.5 cm (30-cm effective length); applied voltage, −22 kV.

may be used to modulate the EOF in order to widen the separation window, to increase the solubility of analytes, or to influence their acid–base properties. As such, for the coated capillary to be useful under practical conditions, it is imperative that it should also exhibit tolerance of these organic solvents. The EOF in KEIO–PB(1.5%) capillary was measured before and after 15-min, high-pressure rinses with some of the commonly employed organic solvents modifiers in CE (used herein as pure solvents), and stability was assessed on the basis of the degradation rate, as shown in Table 1. As can be inferred from the results, degradation is minimal, which further attests to the stability of the coating.

KEIO–PB capillary was also tested against 1 M sodium hydroxide and 0.1 M hydrochloric acid. Being silicate-based, it showed poor tolerance of NaOH, and ~33% reduction in EOF was observed in the run immediately following a 15-min rinse with the base. By comparison, after treatment with HCl, the capillary showed faster EOF. Such observation can be explained partly by a hysteresis effect and partly by a "polishing" effect of the acid;

Table 1. Chemical Stability of KEIO–PB Capillary[a]

	EOF[b] ($m^2 V^{-1} s^{-1}$) ($\times 10^{-8}$)	EOF[c] ($m^2 V^{-1} s^{-1}$) ($\times 10^{-8}$)	degradation rate(%)
methanol	2.15	2.13	0.56
acetonitrile	2.13	2.10	1.01
acetone	2.10	2.07	1.63
2-propanol	2.06	2.05	0.28
0.1 M HCl	2.05	2.40	(17.07)[d]
1 M NaOH	2.40	1.61	32.91

[a] Conditions: KEIO–PB(1.5%) capillary, 38.5 cm (30-cm effective length): BGS, 50 mM ammonium acetate, pH 4.5; applied voltage, −22 kV. [b] Measured EOF before a 15-min high-pressure rinse with solvent/solution. [c] Measured EOF after a 15-min high-pressure rinse with solvent/solution. [d] Please refer to explanation in text.

Table 2. Run-to-Run and Coating-to-Coating Reproducibility[a]

	EOF ($m^2 V^{-1} s^{-1}$) ($\times 10^{-8}$)	%RSD ($n = 10$)
capillary 1	2.16	0.84
capillary 2	2.13	0.73
capillary 3	2.33	0.90
capillary 4	2.24	1.43
capillary 5	1.95	0.49
coating-to-coating ($n = 5$)	2.16	6.48 ($n = 5$)

[a] Conditions are the same as given in Table 1.

Table 3. Performance of KEIO–PB Capillary in CE–MS Analysis of Selected Anions ($n = 5$)[a]

		peak area		migration time (min)	
compound	m/z	average ($\times 10^3$)	RSD (%)	average	RSD (%)
glyoxylate	73	34	2.8	9.78	2.1
glycolate	75	64	3.1	9.47	2.0
pyruvate	87	81	4.0	9.40	2.0
lactate	89	128	3.1	10.19	2.2
fumarate	115	96	3.7	8.05	1.5
succinate	117	229	2.3	8.27	1.6
malate	133	133	6.4	8.25	1.6
2-oxoglutarate	145	161	2.4	8.26	1.6
phosphoenolpyruvate	167	132	3.0	8.38	1.3
dihydroxyacetonephosphate	169	182	5.0	9.47	2.0
glycerophosphate	171	302	3.7	9.63	2.0
3-phosphoglycerate	185	175	4.9	8.46	1.4
gluconate	195	374	4.2	12.19	2.7
erythrose 4-phosphate	199	153	2.4	10.09	2.2
ribulose 5-phosphate	229	398	3.0	10.09	2.2
ribose 5-phosphate	229	539	2.9	10.27	2.0
glucose 1-phosphate	259[b]	861	2.1	10.64	2.3
2,3-diphosphoglycerate	265	92	5.9	8.98	0.7
6-phosphogluconate	275	354	7.4	9.06	1.6

[a] Conditions: KEIO–PB(1.5%) capillary, 100-cm effective length; BGS, 50 mM ammonium acetate, pH 8.5; CE voltage, −30 kV; ESI voltage, −3.5 kV; injection, 30 s at 50 mbar; sheath liquid, 5 mM ammonium acetate in 50:50 methanol/water at 10 μL/min. [b] Data only on peak 1 are given because two compounds comigrated under peak 2.

i.e., it strips the outermost layer of the coating and exposes more of PB on the surface. This stripping is accompanied by removal of some PB as well; thus, coated capillaries treated with HCl and other acids, such as acetic acid and formic acid, evidenced slightly lower long-term stability than those that were not exposed to the same treatment.

(4) Reproducibility. Reproducibilities were assessed based on the relative standard deviations (RSDs) of the EOF obtained in 10 replicate runs, using 5 KEIO–PB capillaries from 5 independent preparations, and the results are shown in Table 2. The run-to-run RSDs were all within 1.5%, while coating-to-coating RSD was within 6.5%.

KEIO–PB Capillary for Analysis of Anions. CE analyses of anions are generally performed under reversed electrode polarity configuration,[40,41] such that the electrophoretic migration of the anions and the EOF are in the same direction, and the analyses can be completed within reasonable time. Using the same configuration in CE–MS, the reversed EOF must be adequately fast and constant to prevent deleterious current drops resulting from the formation of liquid gaps at the exit end of the capillary.[42] EOF reversal can be easily accomplished by addition of cationic modifiers to the running buffer; however, the overall stability of dynamic coating in CE–MS is less than that in CE with UV detection.[42] Accordingly, a capillary in which positive charges are stably fixed to the wall is preferred.

KEIO–PB capillary was employed in CE–MS analysis of some anionic metabolites included in the glycolytic, tricarboxylic acid, and pentose phosphate pathways at pH 8.5, and good reproducibility of data on peak areas and migration times was obtained (Table 3; Supporting Information, Figure S1). It was applied successfully in the analysis of these compounds in the extract of fibroblast NIH 3T3 cells (Figure 5), as well as in rat plasma. The latter was prone to adsorb on the wall and block the charged, active sites; thus, the EOF progressively slowed down in the succeeding runs. However, by rinsing with 0.1 M HCl to flush out the adsorbed materials prior to conditioning with the running buffer, the EOF could be restored (Supporting Information, Figure S2).

In an uncoated capillary, enrichment of anions by prolonged electrokinetic injection is not very straightforward because, under alkaline conditions, the magnitude of the oppositely directed EOF is generally higher than the electrophoretic velocities of the anions. This necessitates the presence of a low-conductivity plug (e.g., water) prior to sample injection and careful monitoring of current for the requisite polarity switch. Using KEIO–PB capillary, field-enhanced sample injection of some anions could be carried out easily (Supporting Information, Figure S3), and more than 2000-fold improvement in detector response was obtained. The same configuration can be carried out using a neutral capillary. However, the use of KEIO–PB capillary has the potential of affording higher enhancement factors by exploiting the benefit of a similarly directed EOF, since the amount of ion injected depends on its velocity at the injection point, which in turn is the vector sum of its electrophoretic velocity and the EOF.

Polymerized Silicate as a Base for Other Coatings. Other cationic polymers can be similarly entrapped in polymerized silicate. For instance, KEIO–DEAE-dextran and KEIO–PEI capillaries were prepared easily, and these showed the same stability

(40) Romano, J.; Jandik, P.; Jones, W. R.; Jackson, P. E. *J. Chromatogr.* **1991**, *546*, 411–421.
(41) Jones, W. R.; Jandik, P. *J. Chromatogr.* **1991**, *546*, 445–458.
(42) Soga, T.; Ueno, Y.; Naraoka, H.; Ohashi, Y.; Tomita, M.; Nishioka, T. *Anal. Chem.* **2002**, *74*, 2233–2239.

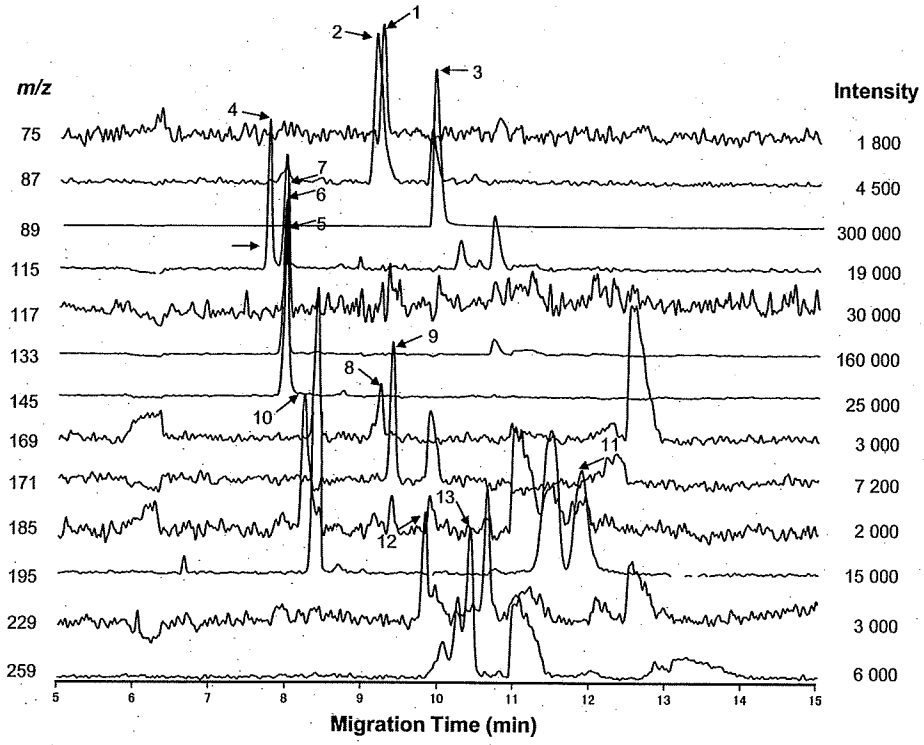

Figure 5. Some anionic metabolites found in the extract of fibroblast NIH 3T3 cells by CE–MS. Peaks (peak number, *m/z*): glycolate (1, 73); pyruvate (2, 75); lactate (3, 89); fumarate (4, 115); succinate (5, 117); malate (6, 133); 2-oxoglutarate (7, 145); dihydroxyacetonephosphate (8, 169); glycerophosphate (9, 171); 3-phosphoglycerate (10, 185): gluconate (11, 195); ribose 5-phosphate (12, 229); fructose 6-phosphate and glucose 6-phosphate (13, 259). Intensity value indicated on the right-hand side refers to that of the most intense peak shown at the given *m/z*. Peaks were detected as their deprotonated ions. Conditions are the same as given in Table 3.

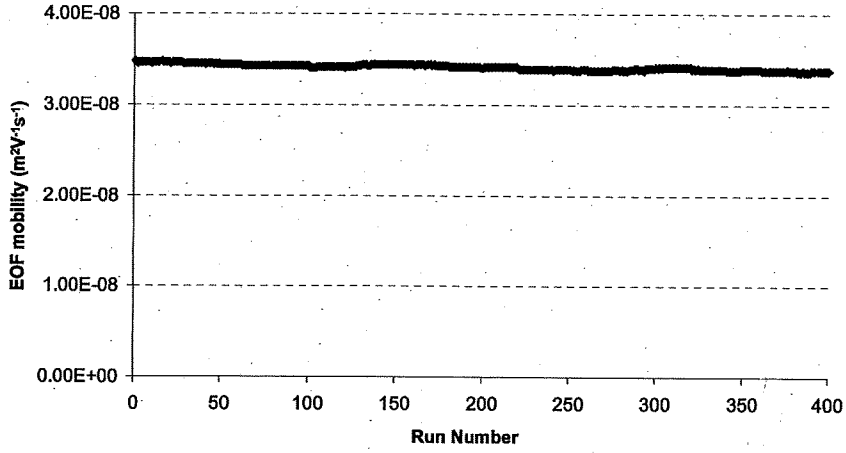

Figure 6. Endurance of KEIO–PB(1.5%)–DS (5%) capillary. Conditions: capillary, 38.5 cm (30-cm effective length); 50 mM phosphate, pH 2.5; applied voltage, 22 kV.

as KEIO–PB capillary, though, at the same polymer concentration (i.e.,1.5%), they exhibited slightly slower EOF. In particular, when KEIO–DEAE-dextran capillary was used under the same conditions for CE–MS analysis of anions as described previously, better separation performance was obtained, and the isomeric pair of ribulose 5-phosphate and ribose 5-phosphate ($m/z = 229$, partially overlapping in Supporting Information Figure S1, $R_s = 0.8$) was baseline separated. Furthermore, fructose 6-phosphate and glucose 6-phosphate (comigrating under peak 2 at $m/z = 259$ in Supporting Information Figure S1) were better resolved ($R_s = 0.9$). KEIO–DEAE-dextran capillary was used continuously in more than 100 analytical runs of real samples without significant degradation in performance.

The KEIO-cationic polymer layer can also be used as a base for attaching anionic polymers on the wall, via electrostatic interaction with the cation in the primary layer. Using KEIO–PB(1.5%), we prepared DS(3%)-coated capillary, which showed a fast, pH-independent, cathodic EOF (Figure 3). KEIO–PB(1.5%)–DS(5%) capillary exhibited excellent endurance, with no appreciable change in EOF in 400 successive runs (Figure 6). By comparison, an equivalent capillary in which the primary layer was attached to the wall, using conventional physical adsorption method, endured only 100 runs.[10] The lower stability of the previous two-layer coating scheme made up of successive layers of oppositely charged polymers was thought to be due to the weak attachment of the inner layer of cations to the silica surface. Thus,

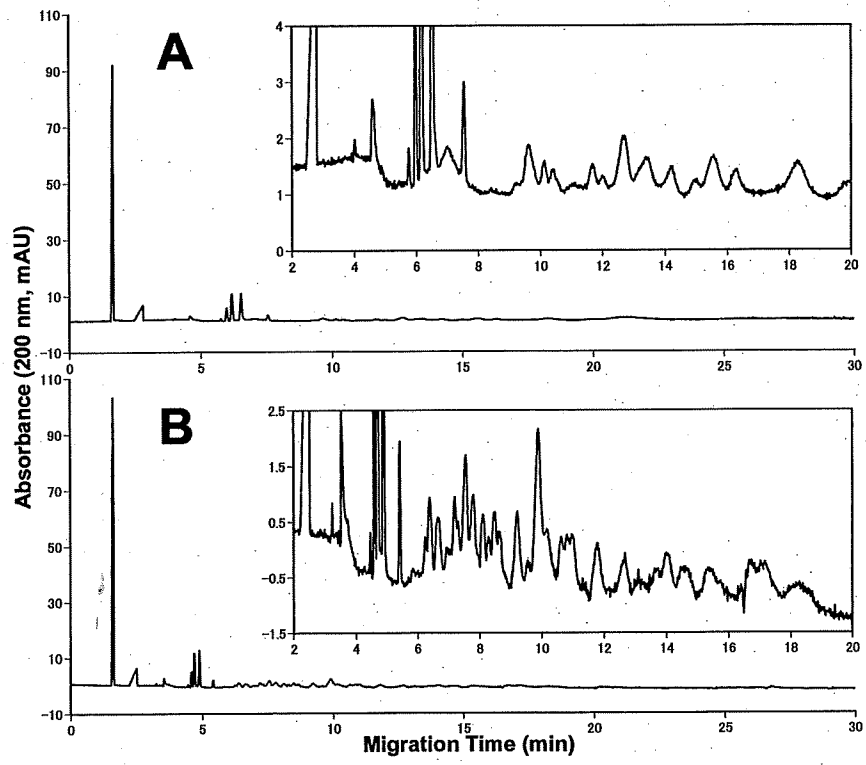

Figure 7. CE analyses of tryptic digest of pig serum albumin using KEIO–PB(1.5%) (A) and KEIO–PB(1.5%)–DS(3%)–PB(10%) (B) capillaries. Conditions: capillary. 58.5 cm (50-cm effective length); BGS, 100 mM phosphate, pH 2.0; injection, 5 s at 50 mbar of 5 pmol/μL digest; applied voltage, −30 kV. Insets show expanded views of the electropherograms between 2 and 20 min.

when this was supplanted with silicate-stabilized cations, the overall stability of the coating was improved. KEIO–PB(1.5%)–DS(5%) capillary was also evaluated for its tolerance of common organic solvents, namely, methanol, acetone, acetonitrile, 2-propanol, and ethanol, as well as 0.1 M HCl, and 1 M NaOH in a manner similar to that described for KEIO–PB. It showed no remarkable change in EOF (i.e., degradation rates were all less than 0.2%) in the runs following high-pressure rinses with any of the abovementioned solvents or solutions. The RSDs of the observed EOF mobility in 10 replicate runs from 5 independent preparations were all better than 0.4%, while the coating-to-coating RSD was within 4.1%. KEIO–PB(1.5%)–DS(5%) capillary showed good performance in the analysis of acidic proteins at physiologic pH, as well as in rapid micellar electrokinetic chromatography-sodium dodecyl sulfate analysis of benzene derivatives at low pH and normal polarity (Supporting Information, Figure S4).

The capillary was further developed by adding a third layer, consisting of cationic polymers adsorbed on the surface of the second layer (KEIO–PB(1.5%)–DS(3%)–PB(10%)). Whereas a one-layer coating has been demonstrated as adequate for small molecules, the presence of unmasked silanols impaired the performance of the capillary for those with tenacious affinity for them, e.g., peptides. Therefore, it was necessary to attach a third layer of PB to more effectively shield the silanols and obtain better results. Figure 7 compares the performance of KEIO–PB(1.5%) and KEIO–PB(1.5%)-DS(3%)–PB(10%) capillaries in the analysis of a tryptic digest of pig serum albumin at low pH. Faster migration times of the peptides in the latter suggest higher density of PB on the surface and presumably better shielding of silanols; thus, sharper peaks were observed.

CONCLUSION

A new, facile strategy for preparing coated capillaries based on polymer entrapment in polymerized silicate was developed. The KEIO–polymer coated capillaries have strong endurance thereby ensuring their long working lifetimes, while their good stability over a wide range of pH and chemical tolerance enables their effective utilization under diverse separation conditions. The coating technique has been demonstrated herein using a conventional capillary format. However, the ease with which the procedure can be carried out makes it easily exportable to the chip format as well.

ACKNOWLEDGMENT

The authors gratefully acknowledge PQ Corporation for the gift of potassium silicate solution, Sachie Sato, Satsuki Ikeda, Yasuyuki Igarashi, and Yuji Kakazu of IAB for technical assistance, and Shota Miyazaki of GL Sciences for the SEM images of the coated capillary. We also thank Prof. Takaaki Nishioka of IAB for support and fruitful discussions.

SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

Received for review May 21, 2007. Accepted July 30, 2007.

AC071038Y

Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography A, 1168 (2007) 237–246
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Review

Metabolome analysis by capillary electrophoresis–mass spectrometry

Maria Rowena N. Monton[a], Tomoyoshi Soga[a,b,*]

[a] *Institute for Advanced Biosciences, Keio University, Tsuruoka, Yamagata 997-0017, Japan*
[b] *Human Metabolome Technologies Inc., Tsuruoka, Yamagata 997-0017, Japan*

Available online 24 February 2007

Abstract

Capillary electrophoresis (CE)–mass spectrometry (MS), as an analytical platform, has made significant contributions in advancing metabolomics research, if still limited up to this time. This review, covering reports published between 1998 and 2006, describes how CE–MS has been used thus far in this field, with the majority of the works dealing with targeted metabolite analyses and only a small fraction using it in the comprehensive context. It also discusses how some of the key features of CE–MS were exploited in selected metabolomic applications.

Keywords: Metabolome analysis; Metabolomics; CE–MS

Contents

1. Introduction

"Metabolomics" is the newest "omics" buzzword in the field of systems biology, joining genomics, transcriptomics and proteomics. The "metabolome," the complete set of small (typically less than 1 kDa) molecules (metabolites) present in cells in a particular physiological or developmental state [1], is said to be closest to the phenotype; thus, metabolomics is poised to play a critical role in understanding intricate biochemical and biological systems. Metabolites are not organism-specific; hence, a single approach for a particular metabolite or metabolite class can be adopted regardless of the species being interrogated [2]. Moreover, their number is projected to be less than those of genes and proteins [1,3]. Despite these, metabolome analysis presents a primary analytical challenge because the wide diversity of metabolites in terms of physico-chemical properties and abundance make their simultaneous determination difficult [4–8]. They can range from small inorganic ions to hydrophobic lipids and complex natural products, and occur in widely diverging concentrations,

DOI of original article:10.1016/j.chroma.2007.02.067.
* Corresponding author at: Institute for Advanced Biosciences, Keio University, Tsuruoka, Yamagata, Japan. Tel.: +81 235 29 0528; fax: +81 235 29 0574.
E-mail address: soga@sfc.keio.ac.jp (T. Soga).

0021-9673/$ – see front matter
doi:10.1016/j.chroma.2007.02.065

spanning over nine orders of magnitude (from pmol to mmol) [7].

Given that the ultimate goal of metabolomics is to identify and quantify *all* metabolites in a given system in an unbiased, reproducible way [2], there is no single analytical methodology that is currently available, which is capable of doing so [2,4–8]. Thus, metabolomics, in the strictest sense, is practically impossible, and the term is used broadly to cover approaches concerned with investigating subsets of the metabolome. These approaches include metabolite profiling, target analysis, metabolite fingerprinting and metabolite footprinting [2,5–9]. Metabolite profiling involves identification and quantitation of a group of metabolites common to a specific pathway or chemical class. Target analysis is even more exclusive in that it focuses only on a particular metabolite or metabolite class. In contrast, metabolite fingerprinting and footprinting involve rapid, comprehensive analysis of samples, generally for the purpose of observing signature patterns, i.e., "fingerprints" or "footprints" (i.e., extracellular metabolites, such as those in spent culture media), without necessarily identifying nor quantifying individual metabolites.

Metabolomics draws on a range of analytical platforms including nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry (MS), and chromatography- and electrophoresis-based separation methods. NMR is an attractive technique for high-throughput fingerprinting and profiling studies as it does not require extensive sample preparation, is non-destructive and can uniformly detect all compounds with NMR-measurable nuclei [10]. Its principal drawbacks, however, are its poor sensitivity and high sample requirement. MS, on the other hand, affords high sensitivity and selectivity, but it can discriminate against some compound classes, depending on the type of ionization used. NMR and MS may be employed as standalone techniques; however, it is generally regarded that strategic matings with upstream separation methodologies enhance their performance for complex mixtures.

Practical issues in interfacing gas chromatography (GC) and liquid chromatography (LC) with MS were resolved early on; hence, they are considered as mature technologies. GC–MS, in particular, is a mainstay in plant metabolomics [11]. A significant development in GC in the last decade is comprehensive, two-dimensional GC (GC × GC). Generally, the sample components are separated in the first column according to their volatilities, then small fractions of the effluent are trapped and focused using a modulator, and sequentially released into the second column for further separation, this time, based on polarity differences [12]. GC × GC offers much greater peak capacities, and with high-scan speed time-of-flight (TOF)-MS as detection method, it is good for complex samples [13,14]. GC, however, is limited to volatile metabolites and those that can be derivatized to yield volatile, thermostable products. In contrast, LC is more rugged and amenable to more types of compounds. Whereas traditionally regarded as inferior to GC in terms of separation efficiency, recent technology advancements in LC have resulted in improved performance. For instance, innovations in pump systems enable operations at elevated pressures, and led to the development of the so-called ultra-performance LC (UPLC). With particles less than 2 μm packed in long capillary columns, fast analyses with as high as 300,000 plates can be obtained [15]. UPLC-MS is used increasingly in metabolomic applications [16,17]. Still, a large number of metabolites are too polar to be significantly retained by reversed-phase columns that are commonly employed [18]. Since a major fraction of metabolites are polar and ionic, a good approach is to use capillary electrophoresis (CE)–MS. In contrast with GC and LC, which operate based on differential interaction with a stationary phase, CE separates analytes according to their mass-to-charge ratios. This orthogonality in separation principle underscores the relevance of CE as a complementary tool to the more established chromatographic techniques—in many cases, samples that cannot be easily resolved by GC or LC can be separated by CE. Fast, highly efficient separations, without requiring rigorous sample pretreatment, can be obtained, and running costs are low. The most attractive feature of CE, though, is its small sample requirement (a few nanoliters at most), making it particularly well-suited for samples that are volume-limited. Poor concentration sensitivity, which is often cited as a disadvantage of CE when fitted with absorbance-related detectors, does not pose a significant problem with MS for detection. Additionally, it is possible to perform facile in-capillary sample enrichment schemes to boost sensitivity [19–22], without the need for dedicated instrumental modifications, when necessary. Thus, CE–MS represents a viable platform for metabolomic studies. To date, however, reports based on CE–MS constitute only a small fraction, with most of these dealing with targeted metabolite analysis, and only a few in the context of comprehensive metabolome analysis. In this review, we describe how CE–MS has been applied thus far in the field of metabolomics, and discuss how some of its key features were exploited in selected applications.

2. Technical considerations

2.1. CE

CE is a versatile technique which is able to separate a wide range of analytes, from small inorganic ions [23] to large proteins [24] and even intact bacteria [25]. By simply incorporating additives (e.g, micelle, chiral selector, complexing agent) into the run buffer, a variety of separation formats can be explored. But because of buffer component constraints when coupling with MS via electrospray ionization (ESI), the most common interface, its simplest form, i.e., capillary zone electrophoresis (CZE), is utilized, with few exceptions. Micellar electrokinetic chromatography (MEKC), a powerful mode for separating neutrals based on partitioning to charged micelles, cannot be combined with MS in a straightforward way because micelles tend to contaminate the ion source, suppress analyte ionization, and decrease MS response. To prevent sensitivity losses, modifications on the MEKC side have been adopted, including the use of volatile [26] and high molecular weight micelles [27], and the so-called partial filling (PF) technique [28], in which separation is effected only in a limited section of the capillary filled with micellar solution. As demonstrated by Frommberger et al. [29], *N*-Acyl-L-homoserine lactones (AHLs), a class of micro-

bial signaling molecules with no chargeable group, could be separated by PF-MEKC-MS, using, at most, 80% of the total capillary length for micellar separation, and two AHLs from *Burkholderia cepacia* colonizing the rhizosphere of traditional Indian rice cultivars could be unambiguously identified. The difficulty with such an approach, however, is that separation performance is often compromised. Thus, interfacing via alternative ion sources [atmospheric pressure chemical ionization (APCI) and atmospheric pressure photoionization (APPI)], while leaving the separation conditions intact, have also been carried out (further discussion on APCI and APPI can be found in Section 2.2).

Derivatization imparts more suitable characteristics to the analytes of interest. It is generally detection-oriented, associated with the incorporation of functional groups to the molecules for enhanced detection sensitivity [30]. In some cases, derivatized moieties can be resolved better. Che et al. [31] subjected dextran to acid hydrolysis, and the hydrolysate was derivatized with 8-aminonaphthalene-1,3,6-trisulfonate (ANTS) by reductive deamination. This introduced negative charges to the oligosaccharides, which facilitated their migration in an electric field. Larsson et al. [32] adopted the same technique for maize starch oligosaccharides, enabling their separation as anions even at low pH conditions. In the preceding examples, the derivatization step was completed prior to sample injection. In a different strategy, Ptolemy et al. [33–35] performed this step within the capillary via zone passing of a concentrated plug of the derivatizing reagent during electromigration. Since derivatization also alters the migration behavior of analytes, this has the additional benefit of enabling sample preconcentration, thereby increasing sensitivity. This technique was applied in analyses of bacterial biomarkers and in fundamental metabolic flux studies in *Escherichia coli* cell cultures.

CE buffers are generally aqueous-based, though nonaqueous systems are exploited as well, particularly for analytes that are insoluble or sparingly soluble in water. Moreover, they permit some hydrophilic interactions, such as hydrogen-bonding, dipole-related and ionic interactions, which are thermodynamically strengthened in hydrophobic environment, to be explored better [36]. When hyphenated with MS via ESI, nonaqueous CE (NACE) circumvents buffer compatibility problems, and even enhances sample ionization process which results in improved detection limits compared to separation in aqueous buffer systems [37]. Bianco et al [38] used NACE to determine glycolalkaloids in *Solanum tuberosum* (potato), and the results were found to be comparable to those obtained using LC–MS. In a follow-up work [39], they utilized the system they developed for comparing the glycoalkaloidal content of transgenic and wild type potato tubers. Sturm et al. [40] and Unger et al. [41] used NACE to determine isoquinoline alkaloids in *Fumaria officinalis* and related phytopharmaceuticals, and naphthylisoquinoline alkaloids in crude extracts from an African *Ancistrocladus* species, respectively. Vuorensola et al. [42] analyzed catecholamines in both aqueous and alcoholic nonaqueous solutions, and found that their separations were more efficient in nonaqueous media than in water.

2.2. MS and interfaces

ESI and matrix-assisted laser/desorption ionization (MALDI) are the most common MS interfaces for biomolecular analyses. ESI enables direct transfer of molecules from the liquid phase to the gas phase [43]; hence, it can be more easily adapted for on-line hyphenation of MS with CE, as well as LC. In contrast, approaches integrating MALDI are essentially off-line [44–46], since it requires a solid matrix. MALDI is a popular technique for proteomics, but it is not as widely employed in metabolomics because of the strong interference from matrix ions in detection of low molecular weight compounds [47].

CE–ESI–MS designs should provide a means for completing the CE electrical circuit for analyte separation, while simultaneously providing an electrical potential to the spray tip. This is generally accomplished using sheath-flow or sheathless interfaces. The first is the most common approach by far because of its robustness and the ease with which it can be implemented. In such configuration, a coaxial sheath liquid, generally a hydroorganic mixture, mixes with the effluent at the exit end of the capillary. Its flow rate and composition can be varied to optimize detection. Several reports have indicated that the type and proportion of organic solvent and volatile acids (e.g., acetic acid and formic acid in the positive ion mode) and bases (e.g., ammonia and trimethylamine in the negative ion mode) affect the intensity of MS signals [48–54] and even influence resolution [55]. For instance, García-Villalba et al. [52] showed that the use of 50:50 v/v 2-propanol/water with 0.1% v/v triethylamine gave the highest signals for the negative ion mode detection of hop acids. On the other hand, Li et al. [53] reported that a pure organic sheath solution (i.e., a mixture of 2-propanol and methanol) offered better stability and sensitivity for lipopolysaccharides (LPS) in *Neiserria meningitidis*. More importantly, it was compatible with both negative and positive ion mode detections, permitting the use of polarity switching in a single CE run.

To ensure a stable electrospray, the sheath liquid is usually introduced at a higher flow rate than the capillary effluent [43]. Because dilution occurs at the mixing point, sensitivity tends to be compromised. This problem can be avoided with sheathless interfaces. Several designs are available, but technical difficulties in configuring them have largely precluded their routine use. Edwards et al. [56] fabricated an electrically porous junction by etching a short section of the capillary with hydrofluoric acid (Fig. 1). The concentration and mass detection limits they obtained averaged 7- and 5-fold lower than those previously reported for some *E. coli* metabolites in CE–MS using a sheath-flow system [57]. Zamfir et al. [58] butted the separation capillary to a commercial nanosprayer needle via an in-house assembled joint, and ESI voltage was applied to the needle using a stainless steel clenching device. This CE–nanoESI–MS system was applied to the systematic screening of complex oligosaccharide mixtures, permitting detection in the pmol level. Schultz and Moini [59] used a split-flow technique, in which a small hole was drilled near the capillary outlet, through which a small percentage of the capillary effluent exited and contacted a metal tube acting as the CE outlet/ESI shared electrode. Its

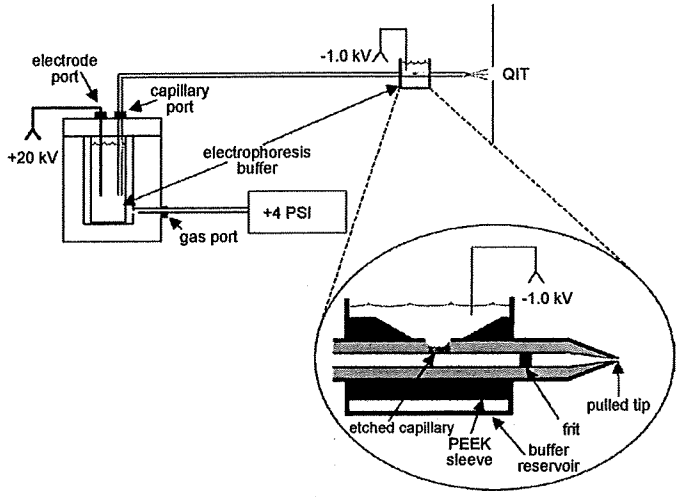

Fig. 1. Diagram of sheathless CE interfaced to quadrupole ion trap MS. Inlet of CE is pressurized for separation using the reservoir shown. Inset shows the etched porous junction. Reprinted from [56] with permission.

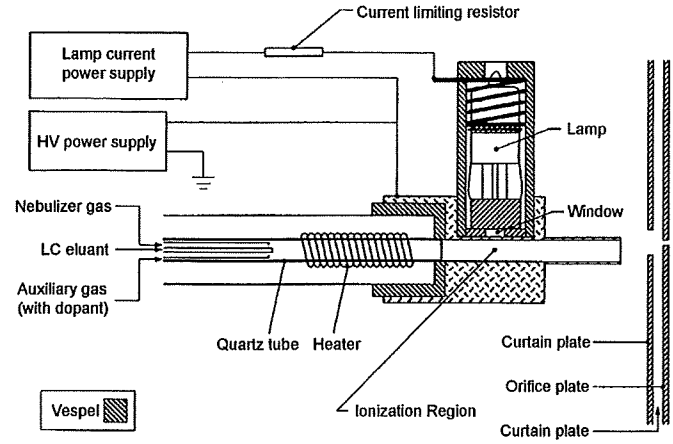

Fig. 2. Schematic of the APPI ion source, including the heated nebulizer probe, photoionization lamp, and lamp mounting bracket. Reprinted from [66] with permission. Copyright 2000 American Chemical Society.

utility was demonstrated by the detection of abnormal levels of amino acids in the blood of newborns afflicted with metabolic disorders.

Based on the principle of the electrospray process, i.e., protonation or deprotonation of solutes or formation of charged adducts, ESI is particularly suited for the analysis of polar compounds [60], but is rather inefficient for nonpolar ones. Moreover, it suffers from low tolerance of salts and susceptibility to matrix effects; thus, good resolution of sample components is important for limiting competing ionizations. With ESI for MS interfacing, the range of CE buffers is restricted to volatile types, which often do not provide the same separation performance as standard buffers for CE with UV detection, such as phosphate and borate. In addition, their concentrations must be kept low so as not to impair ion production, and this generally results in lower efficiencies [61]. By comparison, APCI has been demonstrated to be more tolerant of salts and other nonvolatiles [62,63]. Because ionization conditions are "harder" than those in ESI, it is more suitable for less polar compounds [64]. However, poor sensitivity has deterred its widespread acceptance. It is also not preferred for qualitative assays, such as metabolite identification, because it may produce in-source fragmentation of thermally unstable compounds [65]. APPI [66], a new soft ionization mode, has been shown to be relatively nondiscriminate of nonpolar compounds and reasonably tolerant of matrix additives [67]. The APPI interface (Fig. 2) uses a photoionization lamp and a dopant (a photoionizable molecule, e.g., acetone or toluene) to form dopant radical cations producing protonated eluent molecules, followed by a proton-transfer reaction with the analyte [65]. It has been rapidly adopted in LC–MS [65–69], but few papers regarding its use in CE–MS have been published so far. Nonvolatile buffers [61,70], and even sodium dodecyl sulfate (SDS) [71], the most common micelle used in MEKC, were reported not to affect its ionization efficiency. Thus, with its ability to ionize nonpolar compounds, APPI may be a more widely applicable ionization method for CE–MS. It must be noted, though, that there is an apparent trend toward a "single" ionization source containing combinations of ESI and APCI or ESI and APPI [72].

Almost all types of mass analyzers have been coupled to CE. Because of their relatively low cost, single quadrupoles (Qs) remain popular [32,51,54,57,73–79], though, mainly as mass-selective detectors. Ion traps (ITs) are employed more extensively [21,29,38,40,41,49,50,52,80–89], enabling tandem MS to be performed [21,40,85,86,88] without the need for multiple analyzers. However, the typically narrow peaks resulting from CE separations, in addition to sample complexity, have fueled interest in higher-performance instruments, which are capable of very fast scan speed necessary to adequately describe very sharp peaks, high mass range for expanded coverage, high mass accuracy, high resolution to be able to resolve closely migrating components with similar nominal masses, and/or multi-stage MS capabilities for unequivocal metabolite identification. Thus, triple quadrupoles [22,42,90–94], TOF [76,95–97], and hybrids (e.g., Q-IT [31,56], Q-TOF [58]) are used increasingly.

2.3. CE–MS for comprehensive analyses

CE has always been touted as a high-resolution technique. In principle, the presence of a fast EOF can make all molecules, regardless of charge, migrate in the same direction and be analyzed simultaneously. In combination with MS as detector, the analytical potential is further enhanced because CE enables temporal separation of components that cannot be mass-differentiated (e.g., isomers), while MS provides a second separation dimension for those which co-migrate. Still, highly complex samples cannot be completely characterized because of instrumental limitations related to dynamic range, and analytical parameters, which generally favor the separation and detection of some compounds over others. At best, simultaneous determination of as many components as possible in a single run can be achieved.

For comprehensive analyses, an attractive approach is to use two or more sets of conditions, which have been optimized for different compound groups, and then concatenate the results. In

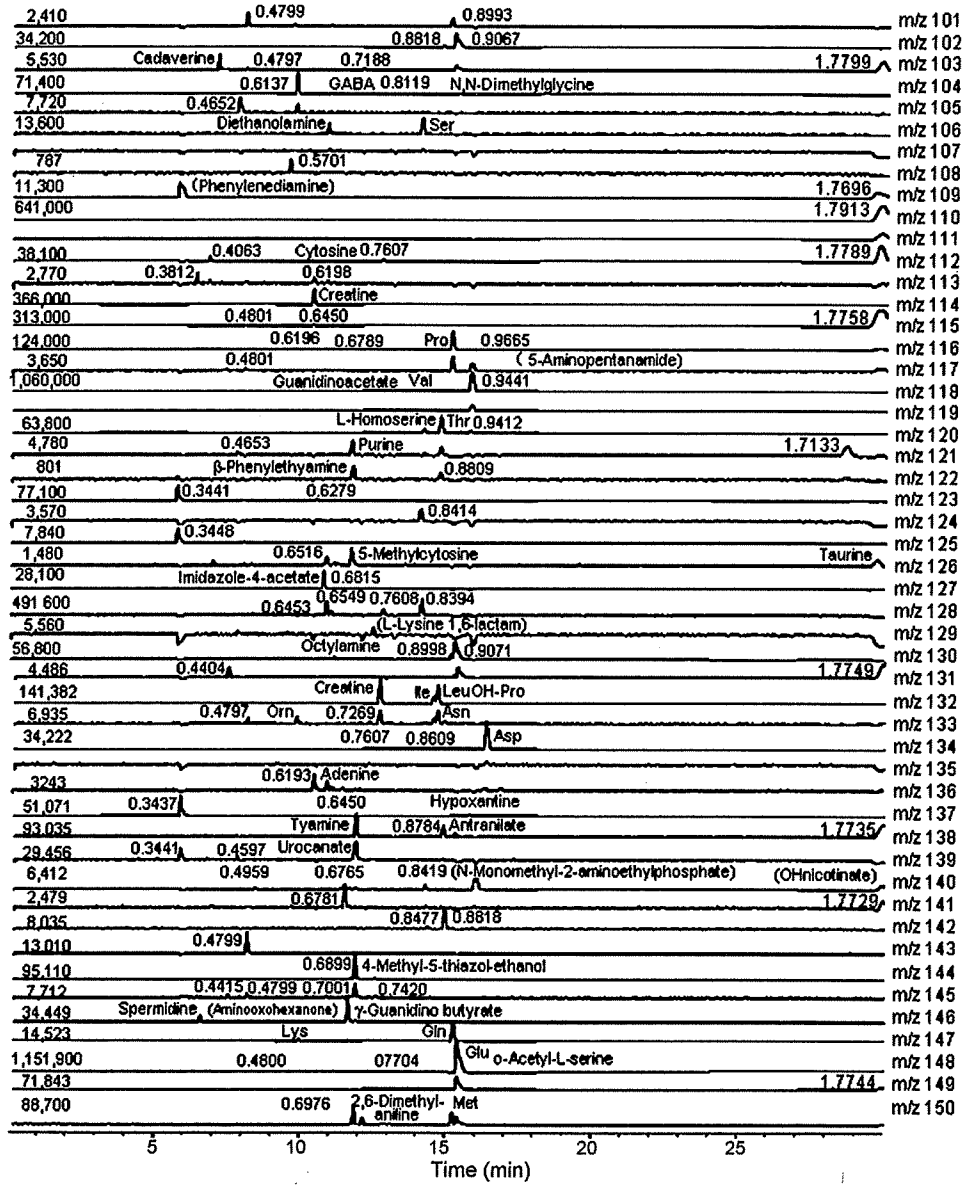

Fig. 3. Selected ion electropherograms for cationic metabolites at $T_{-0.5}$ of B. subtilis 168 in the range of 101–150 *m/z*. The numbers in the upper left corner of each trace are the abundances associated with the tallest peak in the electropherogram, for each *m/z*, and the numbers of tops of peaks are relative migration times normalized with methionine sulfone as internal standard. Reprinted from [79] with permission. Copyright 2003 American Chemical Society.

this manner, wider sample coverage can be obtained, as demonstrated by the works of Soga and colleagues [76,77,79,97]. Amino acids, amines and nucleosides were analyzed as cations using a very low pH electrolyte [54] (Fig. 3). On the other hand, carboxylic acids, phosphorylated carboxylic acids, phosphorylated saccharides, nucleotides, and nicotinamide and flavin adenine coenzymes were analyzed as anions using an alkaline BGS and a cationic polymer-coated capillary (SMILE(+)) to reverse the EOF and prevent deleterious current drops [57]. However, multivalent anions, particularly those of coenzyme A, could not be detected as well-shaped peaks. In a follow-up work [78], they supplanted the SMILE(+)capillary with a neutral capillary in order to prevent anionic species from adsorbing onto the wall, and applied air pressure to the capillary inlet during electrophoresis to provide a constant liquid flow towards the anode. Under these conditions, citrate isomers, nucleotides, dinucleotides, and CoA compounds could be separated and detected well. With a quadrupole mass analyzer, it was necessary to limit the MS scan range to a window of 30 *m/z* in order to maximize detection sensitivity; thus, repeated (33 times, ca. 30 min per run) analysis of the same sample was necessary, requiring as long as 16 h [79]. By switching to a TOF analyzer, however, the number of runs per sample could be reduced to 3 (one for cations, one for anions, one for "nucleotides"). A total of 1692 metabolites from exponentially growing *Bacillus subtilis* cells could be catalogued this way [79] (Fig. 4).

Metabolites are generally identified by matching their migration time and MS or tandem MS spectra against those of pure compounds. However, many metabolite standards are not commercially available (e.g., only 200 of the 600 known

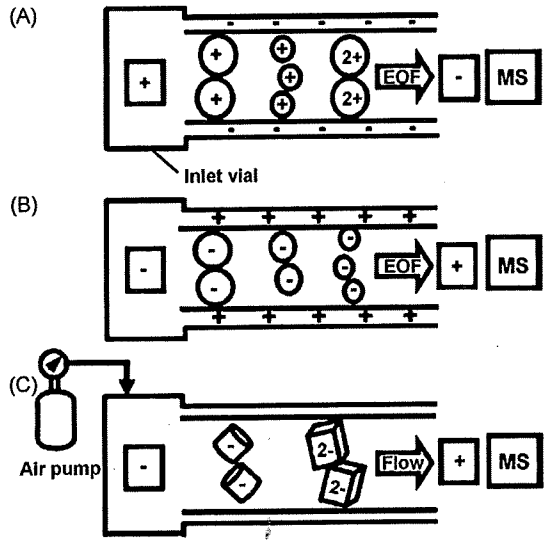

Fig. 4. Schematic of the various CE–MS methods. (A) Cationic metabolites, (B) anionic metabolites, and (C) nucleotides and CoA compounds. Reprinted from [79] with permission. Copyright 2003 American Chemical Society.

yeast metabolites) [8], and metabolomics-specific mass spectral libraries are still limited at this time [7]. For instance, of the 1692 metabolites cited above, only 150 were positively identified and an additional 83 were assigned based on expected charge state and isotopic distribution [79]. For unknown compounds, the use of high-mass accuracy analyzers (e.g., TOF) permit assignment of empirical formulae, while tandem MS enables structural identification via interpretation of their fragmentation patterns. The sheer scale of metabolomics, however, demands novel identification methodologies; as such, computational approaches [21,79,98] are also employed. Sugimoto et al. [98] developed a technique for predicting the identities and migration times of cations in CE–MS using an ensemble of artificial neural networks (ANNs). When evaluated against all metabolites listed in the KEGG (Kyoto Encyclopedia of Genes and Genomes) ligand database, the correct compound among the top three candidates could be predicted in 78.0% of the cases. More recently, Lee et al. [21] reported a method for identifying unknowns by computer modeling of candidate structures to calculate their molecular volumes and intrinsic valence charges, which were subsequently used to estimate their mobilities. Using Simul 5.0 (a freeware), comparisons between predicted and experimental relative migration times of several metabolite candidates gave average relative error under 2%. This strategy enabled differentiation of both isomeric and isobaric amino acid and nucleoside metabolites identified as key nutrients in *E. coli* growth.

For quantitation with ESI–MS, it must be emphasized that most of the uncertainty and potential nonlinearity do not refer to the MS, but rather to the ESI process; thus careful attention has to be given to coupling and separation. An internal standard is the best tool to compensate for changes in ionization efficiency [99].

3. Selected applications

Metabolomic investigations will have strong impact on diverse fields, including genetics, medical science, toxicology, nutrition and agriculture. Their results can be used as chemical basis for taxonomy, to assign functions to genes, identify metabolic intermediates, and elucidate biosynthetic pathways, including novel ones. They can also be used to provide templates for disease diagnosis, or monitor the effects of nutritional and pharmacologic interventions [5]. They can speed the discovery and development of drugs, and to make these drugs safer by predicting the potential for adverse effects earlier [100]. Metabolomics data can be exploited in biotechnology for enhancing the nutritional value of food and engineering of pathways needed for the production of pharmaceuticals in plants [101]. Additionally, they can be applied for assessing the substantial equivalence of genetically modified organisms (GMO) if the metabolic phenotypes of a variety of well-known cultivars, that are commonly believed to be safe, are compared to transgenic plants [102]. Some select applications based on CE–MS are discussed in this section.

3.1. Sample characterization

Several studies involving simple analyses of specific constituents or group of plant principles for which they are valued, were reported [38–41,43,48,75,82,86,103,104], some clearly demonstrating CE as an attractive alternative to LC. Intact, non-desulfated glucosinolates, which have low affinity to reversed phases commonly employed in LC, were analyzed by CE–MS [103]. Using acidic conditions, they were separated and detected as anions, resulting in excellent selectivity and minimal interference from matrix constituents. Additionally, the sensitivity, together with mass accuracy and true isotopic pattern obtainable with TOF-MS, allowed identification of a broad series of glucosinolates in *Arabidopsis thaliana* seeds. Hilz et al. [86] reported better separation of xyloglucan oligosaccharides using CE compared to reversed-phase LC, though separation profiles similar to the former could be obtained by anion exchange chromatography as well. Edwards et al. [82] employed CE–MS for rapid screening of an infusion of aerial parts of *Genista tenera*, a plant used in folk medicine as an antidiabetic agent. At least 26 different phenolic compounds could be distinguished in as short as 10 min, in contrast with a recently reported LC–MS analysis of the same sample where five compounds were separated and identified in 100 min. Furthermore, the use of tandem MS enabled distinction between co-migrating *O*- and *C*-glycosides.

Sato et al. [77] used four independent CE–MS condition tuned for optimum separation and detection of different metabolite classes in the leaves of rice, *Oryza sativa* L. Together with the use of a diode array detector (DAD) almost all water-soluble analytes were determined, and the levels of 88 key metabolites involved in glycolysis, tricarboxylic acid (TCA) cycle, pentose phosphate pathway, photorespiration, and amino acid biosynthesis were measured. Drawing on a similar strategy, Takahashi et al. [105] performed a quantitative comparison of the concentrations of known metabolites, sugars and ions in hygromycin-resistant transgenic rice overexpressing the dihydroflavonol-4-reductase (DFR) gene and transgenic rice with hygromycin-resistant gene alone as control. Differences in the levels of several metabolites, such as *cis*-aconitate, fructose

1,6-bisphosphate, free amino acids, and metals were observed in roots, leaves and seeds, but the concentrations of sugars in seeds were fairly constant.

Carrasco-Pancorbo et al. [49] compared the phenolic contents of different varieties of extra-virgin olive oil. Phenolic compounds in olive oil are acknowledged to be largely responsible for their antioxidant properties, which in turn have been related to their protective effect against chronic and degenerative diseases.

Wahby et al. [88] made the first report about the occurrence of atropine in hemp, *Cannabis sativa*, and determined its concentration, alongside choline, in several transgenic root cultures. Widely varying levels of these compounds were found, and the variations were attributed to differences in plant genotypes, bacterial strains used in inoculation and transformation events. Arráez-Román et al. [80] characterized the methanolic extracts of hop cones, the female flowers of *Humulus lupulus*, which are used for adding flavor and aroma in the beer-brewing process. α-acids were found to be the major components in hops. By analyzing the components present in different extracts, a comparative study of the capacities of different extraction procedures could be carried out. In another work [52], the same group compared the components of different hop varieties before and after natural and forced oxidation in order to determine the best variety as far as storage stability is concerned.

Edwards et al. [56] developed a CE–MS-based method for separation and detection of phosphorylated and acidic metabolites in extracts of *E. coli*, strain DH5-α, enabling 118 compounds to be identified. Interestingly, this figure was higher than those obtained by direct infusion experiments using ESI or MALDI. Li et al. used CE–MS to characterize isomeric LPS of *N. meningitidis* [53] and *Haemophilus influenzae* [106] strains. The occurrence of glycoforms differing by the location or presence of neutral sugar residues was also characterized by tandem MS.

3.2. Elucidating metabolic dynamics

Harada et al. [84] harnessed the usefulness of CE–MS for separation and selective and sensitive detection of underivatized amino acids. ^{15}N-labeled inorganic salts were fed to to *Arabidopsis* (cell line T87) and *Coptis* cultured cells. The ^{15}N labeling ratios of amino acids from T87 cells, cultured under light and dark conditions, at different time points were determined. The rates of increase of the ratios over time were indicative of the length of the pathway for nitrogen incorporation into each amino acid, with most ratios being lower under dark conditions. These results were found to correspond to transcriptional expressions revealed by microarray experiments. In addition, labeling ratios of *Coptis* cultured cells revealed arginine and lysine metabolism inhibition, which should result in inhibition of polyamine biosynthesis and cell division. On the other hand, Tanaka et al. [107] focused on the TCA cycle and its metabolites. The levels of these metabolites in the leaf sheath and leaf blade of transgenic rice expressing the antisense methylmalonate-semialdehyde dehydrogenase, *MMSDH*, were compared to those of control. Results suggested that the concentration of acetyl CoA, the precursor of TCA cycle, is reduced in transgenic rice, and therefore the concentrations of TCA cycle metabolites were altered.

Itoh et al. [108] used CE–MS to perform direct and simultaneous determination of diverse metabolic intermediates for studying the regulation of metabolism *in vitro*. They reconstructed a synthetic *in vitro* glycolysis from ten purified *E. coli* enzymes to obtain a better understanding of the regulation of sequential enzymatic reactions. Their results indicated that the pathway was controlled by a delicate balance between changing metabolite concentrations, and that it behaved like a natural biological oscillating network. Soga et al. [79] employed CE–MS to gain valuable insights into bacterial differentiation and biological events. They profiled *B. subtilis* cells at different time points before and during spore formation stages. Results showed changes in levels of some intermediates in the TCA cycle, such as cis-aconitate, isocitrate, malate, and 2-oxoglutarate, at the end of the exponential growth phase and 2 h thereafter, that were in good agreement with previous findings.

3.3. Assigning functions to genes and proteins

Soo et al. [22] used CE–MS to monitor differences between intracellular pool of sugar nucleotides of parent and isogenic mutants of *Campylobacter jejuni* 81–176. By using product ion scanning, it was possible to determine the precise nature of unexpected sugar nucleotides involved in the biosynthesis of pseudaminic acid. The authors employed sample stacking and obtained as much as 1000-fold increase in sensitivity compared to conventional CE–MS. Additionally, sharper and more resolved peaks were observed under stacking conditions. In a more recent work [109], they used CE–MS in conjunction with hydrophilic interaction liquid chromatography (HILIC)–MS and NMR to investigate the function of flagellin glycosylation genes in *C. jejuni*. They were able to uncover three novel biosynthetic gene functions, completed the structural assignment of two unique glycan-associated intracellular metabolites and demonstrated for the first time *in vivo* a unique interaction between two distinct protein glycosylation pathways.

Saito et al. [76] employed a systematic method based on *in vitro* assays in combination with metabolite profiling to discover novel enzymatic activities. Mixtures of metabolites and candidate proteins were monitored by CE–MS for changes in metabolite composition indicative of the presence of enzymatic activity, while identification of those whose levels changed led to actual substrates and products of reaction. In this way, two proteins from *E. coli*, YbhA and YbiV, were found to display both phosphotransferase and phosphatase activity toward different sugars or sugar phosphates.

3.4. Disease diagnosis and biomarker discovery

Analyses of biofluids, such as urine and blood, can provide important information regarding the metabolic status of an organism. Metabolite concentrations relate to cell and tissue processes, and they reflect both normal variation and toxin- or disease-induced imbalance in single or multiple organ systems

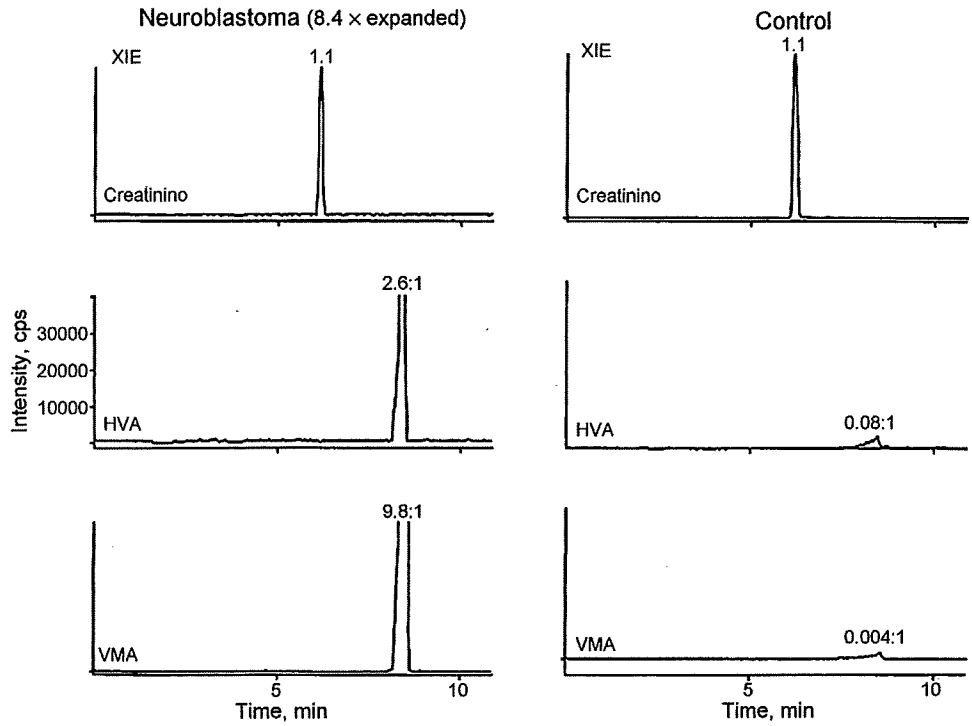

Fig. 5. CE–MS/MS (MRM mode) of a urine sample from a person suffering from neuroblastoma (left) vs. control (right). Reprinted from [90] with permission.

[93]. Some metabolites accumulate as a result of genetic defects causing decreased enzyme activity, thus their abnormally high levels compared to controls can be indicative of diseased conditions, and the information can be used for early detection as well as monitoring progression of a disease. Using CE–MS, it is possible to directly analyze biofluids with minimal pretreatment. Presto Elgstoen et al. [90] described a rapid method for screening for metabolic disorders using urine samples. By using CE–MS/MS in the multiple reaction monitoring (MRM) mode, diagnostic metabolites of several different disorders (e.g., homogentisic acid for alcaptonuria, homovanillic acid (HVA) and vanillylmandelic acid (VMA) for neuroblastoma (Fig. 5), C_{12} and C_{14} epoxy acids for Zellweger syndrome) could be identified. Schultz and Moini [59] applied the technique they developed for analysis of underivatized amino acids in the detection of abnormally elevated levels of phenylalanine and tyrosine in the blood of newborns suffering from phenylketonurea (PKU) and tyrosinemia, respectively.

Ullsten et al. [93] reported a fast and general method for metabolic profiling of urine, combining CE–MS with multi-

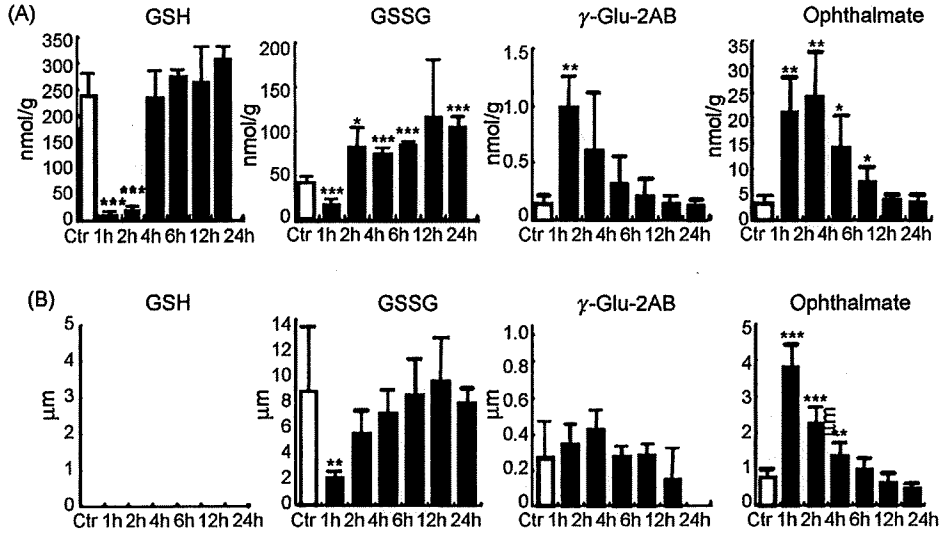

Fig. 6. Changes in metabolite levels in (A) mouse liver and (B) serum 1, 2, 4, 6, 12, and 24 h after acetaminophen (AAP) treatment ($n=4$). *Asterisks* indicate significant differences (***, $p<0.001$; **, $p<0.01$; *, $p<0.05$. The hepatic glutathione (GSH) level at 1 h after AAP treatment was ~28 times lower than in the controls ($p<1.6\times10^{-5}$). On the other hand, the ophthalmate level in mouse serum at 1 h after AAP treatment increased ~5-fold compared with the controls ($p=0.0001$). Reprinted from [97].

variate data analysis. Urine profiles before and after intake of paracetamol could be differentiated, and the *m/z* values of the compounds responsible for the differentiation could be pinpointed. However, since these compounds were not definitively identified, it was not possible to ascribe biological significance to those correlated to ingestion of paracetamol. In contrast, Soga et al. [97] developed a differential display tool with identification capability. Serum and liver extracts of mice before and after acetaminophen-induced hepatotoxicity were profiled by CE–MS, and changes in metabolite profiles, including their levels, were highlighted. In this manner, activation of ophthalmate biosynthesis pathway was revealed, and serum ophthalmate was shown to be a sensitive indicator of hepatic glutathione depletion, and thus, may be a new biomarker for oxidative stress (Fig. 6).

3.5. *Assessing food safety*

The potential for changes in the level of natural toxicants that could result from metabolic processes associated with uptake, biosynthesis and transport of nutritional components, is considered critical in determining the overall risk for food derived from genetically modified plants [39]. Bianco et al. [39] used NACE-MS to compare the steroidal glycoalkaloid (GA) content of transgenic tubers of potato plants, virus Y-resistant, intermediate, susceptible, and control (var. Desiree), and found no statistically significant differences among them. GAs are a class of toxicants involved in the chemical defense of plants, acting as nonspecific protectors or repellents against potential pest predators. They are not destroyed by typical food processing, thus, it is important to monitor their levels in tubers intended for human consumption.

4. Conclusions and future outlook

From the technological viewpoint, metabolomics is a very demanding field. Currently, an "ideal" analytical platform does not exist, and it is unlikely that there will be one in the future. However, continuous improvements in instrumentation that increase the throughput, robustness, sensitivity and accuracy of present methods, in addition to the development of automated tools for processing huge amounts of data, can be expected to expand sample coverage.

Different methodologies have distinct advantages that can be exploited in investigating different metabolite classes, and the resulting information put together to obtain better characterization of the metabolome; thus, the importance of complementary approaches. In this regard, CE–MS definitely has a place in metabolomics research. Though reports up to this time are far outnumbered by GC–MS- and LC–MS-based ones, and most of these deal with targeted rather than comprehensive metabolite analyses, its usefulness for polar analytes has been clearly demonstrated. With the availability of APPI as MS interface, which is able to generate ions from nonpolar compounds and also extend the range of MS-compatible buffer components and additives, CE–MS will be more broadly applicable.

References

[1] S.G. Oliver, M.K. Winston, D.B. Kell, F. Baganz, Trends Biotechnol. 16 (1998) 373.
[2] R. Goodacre, S. Vaidyanathan, W.B. Dunn, G.G. Harrigan, D.B. Kell, Trends Biotechnol. 22 (2004) 245.
[3] Q.-Z. Wang, C.-Y. Wu, T. Chen, X. Chen, X.-M. Zhao, Appl. Microbiol. Biotechnol. 70 (2006) 151.
[4] C. Birkemeyer, A. Luedemann, C. Wagner, A. Erban, J. Kopka, Trends Biotechnol. 23 (2005) 28.
[5] K. Dettmer, P.A. Aronov, B.D. Hammock, Mass Spectrom. Rev. 26 (2007) 51.
[6] S.G. Villas-Bôas, S. Mas, M. Åkesson, J. Smedsgaard, J. Nielsen, Mass Spectrom. Rev. 24 (2004) 613.
[7] W.B. Dunn, D.I. Ellis, Trends Anal. Chem. 24 (2005) 285.
[8] W.B. Dunn, N.J.C. Bailey, H.E. Johnson, Analyst 130 (2005) 606.
[9] J. Nielsen, S. Oliver, Trends Biotechnol. 23 (2005) 544.
[10] Z. Yang, J. Pharm. Biomed. Anal. 40 (2006) 516.
[11] R.D. Hall, New Phytol. 169 (2006) 453.
[12] J. Dallüge, J. Beens, U.A.Th. Brinkman, J. Chromatogr. A 1000 (2003) 69.
[13] M.Z. Özel, Anal. Bioanal. Chem. 382 (2005) 115.
[14] R.M.M. Perera, P.J. Marriott, I.E. Galbally, Analyst 127 (2002) 1601.
[15] J.E. MacNair, K.C. Lewis, J.W. Jorgenson, Anal. Chem. 69 (1997) 983.
[16] Y. Shen, R. Zhang, R.J. Moore, J. Kim, T.O. Metz, K.K. Hixson, R. Zhao, E.A. Livesay, H.R. Udseth, R.D. Smith, Anal. Chem. 77 (2005) 3090.
[17] I.D. Wilson, J.K. Nicholson, J. Castro-Perez, J.H. Granger, K.A. Johnson, B.W. Smith, R.S. Plumb, J. Proteome Res. 4 (2005) 591.
[18] M. Tomita, T. Nishioka (Eds.), Metabolomics: The Frontier of Systems Biology, Springer, Tokyo, 2005.
[19] P. Britz-McKibbin, M.J. Markuszewski, T. Iyanagi, K. Matsuda, T. Nishioka, S. Terabe, Anal. Biochem. 313 (2003) 89.
[20] L. Jia, B.F. Liu, S. Terabe, T. Nishioka, Anal. Chem. 76 (2004) 1419.
[21] R. Lee, A.S. Ptolemy, L. Niewczas, P. Britz-McKibbin, Anal. Chem. 79 (2007) 403.
[22] E.C. Soo, A.J. Aubry, S.M. Logan, P. Guerry, J.F. Kelly, N.M. Young, P. Thibault, Anal. Chem. 76 (2004) 619.
[23] K. Isoo, S. Terabe, Anal. Chem. 75 (2004) 6789.
[24] S.R. Bean, G.L. Lookhart, J.A. Bietz, J. Agric. Food Chem. 48 (2000) 318.
[25] D.W. Armstrong, G. Schulte, J.M. Schneiderheinze, D.J. Westenberg, Anal. Chem. 71 (1999) 5465.
[26] Y. Ishihama, H. Katayama, N. Asakawa, Anal. Biochem. 287 (2000) 45.
[27] H. Ozaki, N. Itou, S. Terabe, Y. Takada, M. Sakairi, H. Koizumi, J. Chromatogr. A 716 (1995) 69.
[28] K. Koezuka, H. Ozaki, N. Matsubara, S. Terabe, J. Chromatogr. B 689 (1997) 3.
[29] M. Frommberger, P. Schmitt-Kopplin, F. Menzinger, V. Albrecht, M. Schmid, L. Eberl, A. Hartmann, A. Kettrup, Electrophoresis 24 (2003) 3067.
[30] J.C.M. Waterval, H. Lingeman, A. Bult, W.J.M. Underberg, Electrophoresis 21 (2000) 4029.
[31] F.-Y. Che, J.-F. Song, R. Zeng, K.-Y. Wang, Q.-C. Xia, J. Chromatogr. A 858 (1999) 229.
[32] M. Larsson, R. Sundberg, S. Folestad, J. Chromatogr. A 934 (2001) 75.
[33] A.S. Ptolemy, P. Britz-McKibbin, Analyst 130 (2005) 1263.
[34] A.S. Ptolemy, M. Le Bihan, P. Britz-McKibbin, Electrophoresis 26 (2005) 4206.
[35] A.S. Ptolemy, L. Tran, P. Britz-McKibbin, Anal. Biochem. 354 (2006) 192.
[36] M.-L. Riekkola, Electrophoresis 23 (2002) 3865.
[37] I. Bjørnsdottir, J. Tjornelund, H. Hansen, Electrophoresis 19 (1998) 2179.
[38] G. Bianco, P. Schmitt-Kopplin, G. De Benedetto, A. Kettrrup, T.R.I. Cataldi, Electrophoresis 23 (2002) 2904.
[39] G. Bianco, P. Schmitt-Kopplin, A. Crescenzi, S. Comes, A. Kettrup, T.R.I. Cataldi, Anal. Bioanal. Chem. 375 (2003) 799.
[40] S. Sturm, E.-M. Strasser, H. Stuppner, J. Chromatogr. A 1112 (2006) 331.