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Application of Capillary Electrophoretic On-Line Sequential Concentration Based on Micelle to Solvent Stacking

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Abstract

To take advantage of micelle to solvent stacking (MSS) to achieve more sensitivity enhancement with enough selectivity, the application of capillary electrophoretic on-line sequential concentration based on the MSS was reviewed. MSS has been coupled to sweeping FASS, FASI and normal EKI. Furthermore, multiple stacking by coupling MSS with more than one stacking method has also been reported. It is more sensitive and selective than solely used of one or two stacking methods and is very suitable for complex and trace sample analysis.

Keywords

Capillary Electrophoresis (CE), On-Line Sequential Concentration, Micelle to Solvent Stacking (MSS)

1. Introduction

Due to its small volume and the narrow optical path, CE suffers from poor concentration sensitivity, especially when on-line UV detection is utilized [1] [2]. To enhance the detection ability of CE, a number of on-line concentration techniques have been developed. These techniques are based on the changes in the analyte migration velocity at the boundary between sample zone and the background electrolyte [3] [4]. Of these techniques, stacking based on association between the analytes and the surfactants are very useful. MSS is a kind of technique based on this principle, in which the sample is prepared in a micellar solu-

tion without an organic solvent or its aqueous solution. Because the organic solvent in the background electrolyte (BGE) affects the micellar interaction with the analytes, the effective electrophoretic directions of the analytes will reverse at the micellar to solvent stacking boundary (MSSB), therefore causing the analytes focusing [5] [6]. It was first introduced by Quirino and co-workers for the stacking of small organic cations [7] and has already been widely used to analyze organic cations and anions in waste water [8], traditional Chinese medicine [9] and biological fluids [10] [11] [12] analysis. Although sensitivity enhancements of 40 - 1000-fold and detection limits of 0.1 - 0.001 mg/L have been achieved with MSS, its selectivity and sensitivity still can't meet the requirements of real sample analysis. To acquire additional detection ability enhancements, sequential capillary electrophoretic stacking by coupling MSS with other CE on-line concentration techniques has been developed. These sequential on-line techniques include coupling MSS with sweeping (sweeping-MSS) [13] [14] [15], field amplified sample stacking (FASS-MSS) [16] [17], field amplified sample injection (FASI-MSS) [18] [19] [20] [21] [22], MSS via normal EKI sample injections with the assistance of micelles [23] [24] [25] as well as field amplified sample injection and sweeping (FASI-sweeping-MSS) [26] [27]. Compared to single used of MSS, the sequential stacking techniques can enhance the sensitivity 2 - 10 more times and these methods can be more selective to specific analytes. The developments of these sequential techniques have been included in the review of Yang *et al.* [28]. But it is not comprehensive and the works of these techniques after 2013 were not included. In this paper, the application of sequential on-line concentration methods based MSS was reviewed.

2. Sweeping-MSS

Recently, a novel two-step stacking strategy of sweeping-MSS was introduced. In such strategy, the sample matrix was free of the micelles and a plug of anionic (cationic analytes) or cationic (anionic analytes) micellar solution was injected before sample solution in order to perform sweeping [13]. After the sweeping step, the enriched analyte bound to the micelles were transported to the MSSB where they experienced an organic solvent in the BGE. The solvent reduced the interaction between the micelles and the analytes. The direction of the effective electrophoretic mobility reverses at the MSSB. This caused the focusing of the analytes at the MSSB. Compared to single MSS stacking, sweeping-MSS can enhance the sensitivity of analytes 2 - 5 times further. Sweeping-MSS was first introduced by Quirino to determine four β-blockers and two tricyclic antidepressant drugs with capillary zone electrophoresis (CZE)separation [13] and now they have been widely used in organic cations [13] [14] [15] and anions [29] [30] analysis. Yang *et al.* developed a sweeping-MSS technique in CZE and applied this method to determine quinolizidine alkaloids in traditional Chinese medicines. Under the optimum conditions, the sensitivity enhancement factors (SEFs) obtained by the developed method for the analytes were in the 42 - 52

fold range [14]. Yang *et al.* proposed a sweeping-MSS method for fast stacking of nitroimidazoles in CZE. With this method, dimetridazole, metronidazole and secnidazole can be determined in 9 min. The detection limits and sensitivity enhancements are in the 2.3 - 3.0 ng/mL and 12 - 38-fold range, respectively [15]. Yang *et al.* developed a sweeping-MSS on-line concentration method in CZE to determine strychine and brucine in traditional Chinese herbal medicines. The detection limits for the two analytes were 0.01 μg/mL. Compared with typical CZE, 38 - 51-fold sensitivity enhancement were achieved [31]. Quirino *et al.* have developed a sweeping-MSS method using cationic cetyltrimethylammonium micelles in CZE to determine hypolipidemic drugs, herbicides and non-steroidal anti-inflammatory drugs. In their work, the direction of electroosmotic flow (EOF) was the same as the anions which were achieved by positive dynamic coating of a fused silica capillary with hexadimethrine bromide. The sensitivity enhancements based on peak height were in the 17 - 33-fold range. The limits of detections (LODs) were from 0.05 - 0.55 μg/mL range [29]. Wang *et al.* developed a long-chain ionic liquid (LCIL)-based sweeping-MSS method in CZE for anionic compounds analysis. In their work, N-Cetyl-N-methylpyrrolidinium bromide (C_{16}MPYBr) was used as a novel cationic surfactant. The capillary column was conditioned with poly (1-vinyl-3-butylimidazolium) bromide, a kind of polymeric ionic liquid, to obtain the anodic EOF. Under the optimized conditions, the peak areas of benzoic acid and 2-nitrophenol were increased 34 and 25 times and the peak height of 4-chlorophenol was increased 60 times [30].

3. Micelle to Solvent Stacking via Sample Injection under Field Enhanced Conditions

When the conductivity of the sample zone is much lower than the BGE zone thus electric field of the sample zone is much higher than that of the BGE when the voltage is applied. As a result, the analytes sped down and accumulated at the boundary between the sample zone and the BGE. When the sample was introduced with pressure, it is called field amplified sample stacking (FASS). When the sample was introduced electrokinetically, it is called field amplified sample injection (FASI) [32]. MSS relies on the transport of the micelle bound charged analyte and the reversal of the analytes' effective electrophoretic mobility at the MSS boundary between the micellar zone and the adjacent zone [7]. When coupling MSS with FASS, the sample was solved in a low conductivity micellar solution (S). A solution with low conductivity containing large proportion of organic solvent was used as trapping solution (TS). After the capillary was preconditioned with the running buffer, S and TS were sequentially introduced. When the positive separation voltage was applied, the analytes loaded by the negatively charged sodium dodecylsulfate (SDS) micelles will continuously fall down into the organic solvent in the TS, in which the concentration of SDS is below the critical micelle concentration (CMC), consequently releasing and accumulating the transported molecules. The analytes are released into the organic

solvent zone with a low conductivity in which a high electric field is provided, resulting in a high analyte migration velocity. When the ionized analytes arrive at the boundary between the organic solvent zone and BGE zone, the electric field strength suddenly decreases, and the migration velocities of the analytes become slower resulting in the gathering of the analytes near the boundary [16]. As the MSS step is before the FASS step, this sequential method is called micelle to solvent stacking-field amplified sampling stacking (MSS-FASS). But when the conductivity of TS is higher than the sample matrix and the EOF is negligible, the organic solvent in the TS solution can't react with the micellar first. On the contrary, the SDS micelles in the sample zone rapidly moved into TS under high electric field intensity. Due to the electric filed strength of TS is lower than that of sample solution, the analytes slowed down quickly and accumulated at the border between sample zone and the TS zone, which is a FASS process. Thereafter, once the mixed micelles have access into the organic solvent zone, the micelles would collapse and release the analytes, in which a MSS process occurred. As the FASS step is before the MSS step, this sequential method is called micelle to solvent stacking-field amplified sampling stacking (FASS-MSS). Liu *et al.* have poposed a MSS-FASS method in CZE to determine trimethoprim and sulfamethoxazole in animal-originated foodstuffs. LODs of 7.7 - 8.5 ng/mL were achieved which were 301 and 329 times better compared to a typical injection, respectively [16]. Wan *et al.* [17] have developed FASS-MSS method with the aid of β-cyelodextrin (β-CD) to determine the contents of atenolol and metoprolol in human urine by CE. Improvement in the sensitivity for analytes is achieved up to 200-fold compared with conventional CE method. The LODs can be reached 3.3 and 3.7 ng/mL, respectively.

In FASI-MSS, the conductivity of the sample solution is much lower than that of the BGE and the micellar solutions. There are big amounts of organic solvents in the sample matrix. After preconditioning the capillary with the BGE, a micelle solution (MS) plug was introduced with hydrodynamic injection. The sample solution was introduced with hydrodynamic injection for a short time to enhance the electric field strength at the injection point during FASI (Sometimes this step can be omitted, as sample eluent introduced via the low EOF during FASI can also function for this purpose). Then the sample was electrokinetically injected for a long time. The analytes electrophoretically migrate to the positive (anions) or negative (cations) electrode while the micelles travel toward the opposite end. The analytes become trapped in the positively (anions) or negatively (cations) charged micelles, and the micelle-bound analytes travel toward the negative (anionic analytes) or positive (cationic analytes) electrode. The organic solvent present in the sample matrix decreases the affinity of the analytes to the micelles, leading to the release of the analytes from the micelles. Analyte focusing occurs at the MSSB and then the analytes are separated by CZE [18] [19] [21] or nonaqueous capillary electrohporesis [20]. As shown in **Table 1**, compared to Sweeping-MSS, MSS-FASS and FASS-MSS, more analytes can be

Table 1. Comparison between sweeping-MSS and coupling field enhancement with MSS.

Stacking techniques	Stacking mechanism	Sample diluent	Sample clean-up	SEF
Sweeping-MSS	Association between analytes and micelle, collapse initiated by organic solvent in BGE	Same kind of buffer as BGE without micelle	Need sample clean-up process	12 - 60 [14] [15] [29] [30] [31]
MSS-FASS	Micelle collapse initiated by organic solvent in TS, high electric field created by organic solvent in the TS	Micelle solution	Need sample clean-up process	301 – 329 [16]
FASS-MSS	The conductivity difference between low conductivity sample and the TS, micelle collapse initiated by organic solvent in TS	Micelle solution	Need sample clean-up process	200 [17]
FASI-MSS	High electric field created by organic solvent in the sample solution, micelle collapse initiated by organic solvent in sample diluent	Low conductivity sample diluent containing large amount of organic solvent	Need sample clean-up process and the sample amount is vulnerable to sample ionic strength changes	200 - 2534 [18] [19] [20] [21] [22]

introduced in FASI-MSS, which can permit much bigger sensitivity enhancements. Besides, it is the organic solvent in the sample matrix rather than that in the BGE or TS that initiated the MSS process in FASI-MSS. Although these stacking techniques all need sample clean-up, the stacking efficiency is more vulnerable to ionic strength changes in the sample matrix for its low conductivity requirement. Rabanes *et al.* have developed a FASI-MSS method in CZE to determine cationic drugs in urine. Using a sample diluent with one-tenth the conductivity of the background solution, the strategy afforded as high as 2479-fold improvements in peak height and LODs of as low as 1.1 - 1.8 ng/mL [19]. Tubaon *et al.* developed a FASI-MSS method in CZE to determine anionic sulfonamides in river water samples. Cationic cetyltrimethylammonium bromide (CTAB) was used as the surfactant. Compared to typical hydrostatic injection, SEFs of 397 - 1024 have been achieved. Limits of quantitation were in the 0.01 - 0.3 μg/mL range [18]. Thang *et al.* has developed a FASI-MSS method in nonaqueous capillary electrophoresis to determine anticancer drugs in major metablites. The proposed method allowed a 214 - 625-fold SEF which is 2 - 5 times better than with FASI only [20]. Mohd Azmi *et al.* [21] have developed and validated a FASI-MSS method to determine paraquat and bromhexine in river water. The sensitivity was enhanced 1000-fold in comparison to normal CZE. Chong *et al.* [22] developed a FASI-MSS method in the microchip electrophoresis (MCE) coupled with on-chip contactless conductivity detection (C^4D) system to determine vancomycin in human plasma. A SEF of up to 217-fold was achieved and the LODs of the method for vancomycin was 1.2

μg/mL.

4. Micelle to Solvent Stacking via Normal Electrokinetic Injection

If there is no apparent difference in conductivity between the sample zone and the BGE, the samples can be introduced with the aid of micelles, during which the effects of EOF can be attenuated by introducing acidic buffer solution [23] [24] or with the assistance of pressure during EKI [25]. Dong *et al.* combined MSS with large amount sample electrokinetic stacking injection (LASEKSI) in CZE for the analysis of cationic molecules. In this developed MSS-LASEKSI method, after a large amount of sample solution was introduced from the outlet hydrodynamically, co-solvent buffer was injected with pressure from the inlet. And then sample stacking was performed by applying a 10 kV voltage in the normal polarity through the acidic buffer solution (pH 3.0) vial and the sample vial for a period of time, during which the integral EOF was attenuated, and when the velocity of EOF and the micelles are equal, a equilibrium state was formed and can be maintained for a long time, leading to the continuous stacking of the analytes on the basis of MSS. Therefore, an extremely large amount sample was permitted to be injected into the capillary which allowed as high as 6.3×10^3-fold sensitivity enhancement [23]. The developed method has been applied to determine berberine and theophylline in urine samples with recoveries in the range of 98% - 101% and 95% - 112%, respetively. Wuethrich and Quirino proposed the unusual stacking of cationic analytes via EKI of SDS micelles into a fused silica capillary filled with acidic BGE with 40% - 50% acetonitrile. Two peaks were observed from an analyte which suggested the concentration of analytes into two stacking zones. The zones were identified as the SDS micelles (micelles zone) and organic solvent-rich stacking zone (solvent rich zone) where the micelles zone was closer to the inlet end of capillary. The concentrated analytes in the micelles zone were from the concentrated analytes that electrophoretically migrated into the micelles zone from the solvent-rich zone during EKI. The analytes in the micelles zone were then re-stacked by MSS and formed the second sharp peak in CZE. This can be prevented by reduction of acetonitrile concentration in the inlet BGE. SEFs of more than 100 times were obtained for diphenhydramine and imipramine, two cationic drugs [24]. Quirino and Aranas have coupled simultaneous electrokinetic and hydro dynamicinjection (SEHI, also named pressure assisted electrokinetic injection) of organic cations with MSS in reversed migration MEKC to determine tricyclic antidepressant and beta blocker drugs in micellar electrokinetic chromatography. As the pressure counteracted the slow moving EOF and maintained the MSSB inside the capillary relatively stable during injection, much more sample ions can be introduced into the capillary which allowed up to 4000-fold sensitivity enhancement [25]. The method has been applied to determine tricyclic antidepressant and beta blocker drugs in waste water samples with recoveries in the

81% - 87%, range.

5. MSS Based Multiple Stacking

Coupling MSS with more than one stacking method, which is called MSS based multiple stacking, can further enhance the sensitivity and selectivity. In Sweeping-MSS, if the sample was injected with FASI, triple stacking by coupling FASI, Sweeping and MSS (FASI-sweeping-MSS) can be achieved. Grochocki *et al.* have developed a FASI-sweeping-MSS triple stacking method in CZE to determine four penicillins in plasma samples [26]. In their work, the sample was solved in diluted BGE and introduced into the capillary by FASI at negative voltage. Then the CTAB micelles were introduced at positive voltage and the the positively charged micelles swept the stacked anionic analytes at the sweeping boundary. And then a small plug of 60% methonal was introduced hydrodynamically, the MSSB was created between the MeOH and swept analyte zone with micelles. As the negative separation voltage was applied, it resulted in the stacking of the analytes at the MSSB. The SEFs were 146 - 279 and 519 - 954 for conductivity ratio of 10 and 100, respectively. The SEF enhancement factors were 16 - 32 and 6 - 10 times better than solely use of FASI. Recoveries of analytes under different concentration levels were in the 72% - 101% range. Grochocki *et al.* [27] have developed a three-step stacking procedure of combining FASI with sweeping and MSS using model cationic drugs. The sensitivity enhancements of this three-step stacking techniques were in the 308 - 891, 2188 - 6463 and 3088 - 6499-fold range, when the conductivity of sample diluent is 10,100, and 1000× lower than the BGE, respectively. The method has been applied to plasma sample analysis with recoveries in the 81% - 124% range.

6. Conclusion

In this paper, the application of capillary electrophoretic on-line sequential concentration techniques based on the MSS has been discussed from four aspects, *i.e.*, sweeping-MSS, field enhancement-MSS, MSS via normal EKI and MSS based multiple stacking. Compared to MSS, sweeping-MSS is more selective, but the sensitivity enhancements are often below one hundred, possibly due to limited sample amount. As much more analytes can be introduced into the capillary, combination of field enhancement with MSS can obtain more sensitivity enhancement and SEF as high as more than one 2000 has been achieved. When coupling MSS with normal EKI, if the EOF was modulated by pressure or acidic buffer, very large amount of analytes can be injected with the aid of micelle and as high as more than three magnitude of sensitivity enhancements can be achieved. MSS based multiple stacking is much more selective and sensitive and can be used to determine trace analytes in complex sample matrix. As shown in **Table 2**, the application of MSS based sequential stacking techniques has been limited to traditional Chinese medicine, plasma, water and urine sample analysis, which may be due to the limited sample clean-up techniques.

Table 2. Application of MSS based sequential CE stacking techniques.

Analyte	Sample	LOD (ng/mL)	SEF	Combined technique	Reference
Alprenolol, propranolol, nadolol, labetalol, nortriptyline, clomipramine		20	20 - 46	Sweeping-MSS-CZE	13
Sophocarpine, matrine and oxymatrine	Traditional Chinese medicines	20 - 30	42 - 52	Sweeping-MSS-CZE	14
Dimetridazole, metronidazole, secnidazole	Rabbit plasma	2.3 - 3.0	12 - 38	Sweeping-MSS-CZE	15
Gemfibrozil, fluvastatin, atorvastatin, naproxen, diflunisal, ketoprofen, indomethacin, indoprofen, mecoprop and fenofrop	Waste water	50 - 550	17 - 33	Sweeping-MSS-CZE	29
Benzoic acid, 2-nitrophenol, 4-chlorophenol	Tap water and Yellow river	25 - 250	25 - 60	Sweeping-MSS-CZE	30
Strychnine and brucine	Traditional Chinese medicines	10	38 - 51	Sweeping-MSS-CZE	31
Trimethoprim, sulfamethoxazole	Animal-originated foodstuffs	7.7 - 8.5	301 - 329	FASS-MSS-CZE	16
Atenolol, metoprolol	Urine sample	3.3 - 3.7	200	FASS-MSS-CZE	17
Sulfamerazine, sulfamethazine, sulfamethizole	River water	10 - 30	397 - 1024	FASI-MSS-CZE	18
Quetiapine fumarate, droperidol, risperidone, olanzapine, chlorpromazine hydrochloride, aripiprazole	Human urine	1.1 - 1.8	717 - 2479	FASI-MSS-CZE	19
Tamoxifen and its metabolites	Human plasma	0.15 - 0.70	214–625	FASI-MSS-NACE	20
Paraquat and bromhexine	River water	30 - 50	215 - 2534	FASI-MSS-CZE	21
Vancomycin	Human plasma	1200	83	FASI-MSS-MCE	22
Berberine and theophylline	Human urine	0.3 - 0.4	6.4×10^2 - 6.3×10^3	MSS-LASEKSI-CZE	23
Imipramine hydrochloride and diphenhydramine hydrochloride		15	100	EKI-MSS-CZE	24
Imipramine hydrochloride, clomipramine hydrochloride, doxepine hydrochloride, nortriptyline hydrochloride, alprenolol hydrochloride, labetalol hydrochloride and propranolol hydrochloride	Wastewater and urine	0.6 - 4.2	4000	SEHI-MSS-MEKC	25
Penicillin G, oxacillin, ampicillin, amoxicillin	Plasma	6.6 - 13.2	519 - 954	FASI-sweeping-MSS-CZE	26
Dibucaine, diphenhydramine, propranolol, verapamil	Plasma	10 - 40	3088 - 6499	FASI-sweeping-MSS-CZE	27

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Abbreviations

β-CD: β-Cyclodextrin
BGE: Background Electrolyte
CMC: Critical Micelle Concentration
CTAB: Cetyltrimethylammonium Bromide
CZE: Capillary Zone Electrophoresis
EKI: Electrokinetic Injection
EOF: Electroosmotic Flow
FASI: Field Amplified Sample Injection
FASI-MSS: Field Amplified Sample Injection-Micelle to Solvent Stacking
FASI-Sweeping-MSS: Field Amplified Sample Injection-Sweeping-Micelle to Solvent Stacking
FASS: Field Amplified Sample Stacking
FASS-MSS: Field Amplified Sample Stacking-Micelle to Solvent Stacking
LASEKSI: Large Amount Sample Electrokinetic Stacking Injection
LOD: Limit of Detection
MCE: Microchip Electrophoresis
MEKC: Micellar Electrokinetic Chromatography
MS: Micelle Solution
MSS: Micelle to Solvent Stacking
MSSB: Micelle to Solvent Stacking Boundary
MSS-FASS: Micelle to Solvent Stacking-Field Amplified Sample Stacking
MSS-LASEKSI: Micelle to Solvent Stacking-Large Amount Sample Electrokinetic Stacking Injection
SDS: Sodium Dodecyl Sulfate
SEF: Sensitivity Enhancement Factor
SEHI: Simultaneous Electrokinetic and Hydrodynamic Injection
Sweeping-MSS: Sweeping-Micelle to Solvent Stacking
TS: Trapping Solution